

nature

26 October 1978

Is proliferation unstoppable?

In a mere eighteen months' time the Non-Proliferation Treaty (NPT) will come up for its second review conference. The treaty, which came into force in 1970, is intended to prevent non-nuclear-weapon states building their own nuclear devices or receiving them from any other source; in return nuclear-weapon states promise not to transfer nuclear explosives, nor to help others build their own. A majority of all nations adhere to the treaty, but there are a number of potentially nuclear countries who have kept out. West Germany, Japan, Sweden and Switzerland are in, but Argentina, Brazil, France, India, Israel, Pakistan, People's Republic of China and South Africa are not. This latter group contains, of course, three nuclear states, two of which are widely regarded as virtually nuclear and others which could harbour nuclear-weapons ambitions. So the NPT could hardly be said to be an unqualified success.

The first review of the treaty came up in 1975 and was not a happy occasion. The superpowers wanted to emerge unscathed from the conference and were largely successful. The price for this, however, was growing dissatisfaction amongst non-nuclear-weapons states, notably Mexico and Yugoslavia, at the way their self-denial had met with no real promises of restraint on vertical proliferation. In the event no-one withdrew from the treaty, but on the other hand no outsider proceeded to sign.

The 1980 conference could be much more of a cross-roads. Certainly the superpowers will bring a new Strategic Arms Limitation Treaty, and, most likely, a short-duration Comprehensive Test Ban as evidence of self-restraint, though both of these will be heavily criticised. But the real issues will probably be elsewhere. In the past few years, security of future energy supply has tended to supplant other world concerns, including nuclear proliferation. So uranium enrichment, reprocessing and breeder reactors are likely to be topics of importance at the 1980 conference, and the central debate could well focus on the right of nations to have access to these facilities. The Stockholm International Peace Research Institute, SIPRI, has recently held a conference to look at the control of fissionable materials in non-military applications, with a view to stimulating thought on these matters during the run-up to 1980. There are clearly many areas of great concern. Traditional methods of uranium enrichment could be challenged soon by jet-nozzle and vortex-tube techniques in West Germany and South Africa, as well as by laser separation methods. Reprocessing facilities capable of supplying plutonium are being developed in several countries with an eye on export and with the apparent justification of the Non-Proliferation Treaty itself, whose Article IV urges states to cooperate in the further development of peaceful uses of nuclear energy. The technological momentum of breeder research and development is making that device look like the logical next step for some nations, and it has the appeal of enormous savings on resources. Enrichment, reprocessing and breeding all look attractive in the absolute, but all open new doors to the more widespread circulation of weapons-grade materials.

What, the SIPRI meeting asked, is to be set against these proliferative trends? The answer seems to be that no one measure, whether technological, political or institutional

can in itself stem the tide for long; the best that can be hoped for is that by applying as many restraints as possible and in as many ways as possible, non-nuclear countries considering the acquisition of nuclear weapons delay long enough for new attitudes to have emerged, both towards proliferation and towards the role of nuclear energy in national energy strategies. Amongst subjects discussed were the possibility of the International Nuclear Fuel Cycle Evaluation (INFCE) coming up with something valuable; the chances that an Advanced Converter Reactor development in the United States would lead to efficiencies which made a breeder reactor seem unnecessary, at least for the present; possible use of denatured fuel; moratoria on various enrichment technologies and problems posed by fusion-fission hybrids, particularly using inertial confinement techniques. And yet the question ultimately is not technological but political and institutional. Energy independence has become a target, and with many years of almost unbridled enthusiasm for the atom not only in national but also international agencies there is much to be done before new attitudes emerge.

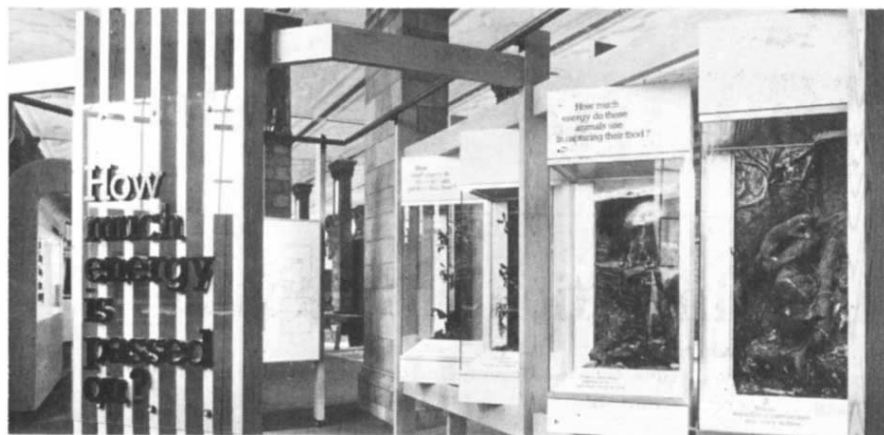
The most overtly political and apparently progressive move so far—President Carter's 1978 Non-Proliferation Act—has not been well-received everywhere. The Act puts strong controls on trading of nuclear materials and equipment originating in the United States, but in exchange tries to assure adequate safeguarded supplies to those countries which co-operate and which have ratified the NPT. Carter has also, of course, been running down domestic development of reprocessing plants and plutonium breeders. Some nations well along the nuclear road have felt this legislation to be discriminatory and unreasonable, even to the extent of wondering whether the United States, whose breeder development was in any case lagging, was not adopting a pious stance based simply on commercial opportunism. In truth Carter's proliferation concern runs much deeper than that, but the last year has shown how unfortunate it is that commercial and national interests are so deeply woven into a fabric already criss-crossed with technological and international problems.

An idea put forward at the SIPRI meeting by A. R. W. Wilson, of Australia, could provide a way out of this bind. He proposes a nuclear fuel supply co-operative of states dedicated to meeting each others' fuel and service requirements whilst minimising the risk of proliferation. Each member would undertake to agree safeguards with the International Atomic Energy Agency (IAEA) and to restrict its access to enrichment and reprocessing facilities, whether on its own or another country's territory, simply to that amount of fuel deemed necessary by the IAEA for the country's power programme. Internationally controlled facilities would not be excluded from the co-operative's activities. The emphasis in the co-operative would be on mutual benefit rather than on surrender of national sovereignty.

This deserves extensive discussion. Much of the criticism of current non-proliferation measures is their sharply discriminatory character; Dr Wilson's proposal would represent a move towards benefit-sharing and should be widely attractive. □



The new exhibition schemes at the British Museum (Natural History) and the consequent changes in the more traditional exhibits have caused some consternation amongst interested scientists. At the recent symposium on Vertebrate Palaeontology and Comparative Anatomy held at Reading, Dr R. S. Miles, Head of the Public Services Department of the Museum explained the Museum's present policy towards its public exhibitions, and we present a summary of his talk below. Dr B. Halstead of the Department of Geology, University of Reading presents an opposing view (right).



A new exhibition on ecology (see above) opens this week . . .

The public's right to know

THE policy of the Public Services Department about what we do in the galleries is not the whim of any one person in the Natural History Museum. It originates with the 1753 Act of Parliament for the purchase of the Hans Sloane Collection. The British Museum Bill says: "The said Museum or Collection may be preserved and maintained, not only for the Inspection and Entertainment of the learned and the curious, but for the general Use and Benefit of the public." The Bill, in other words, distinguished between research and public functions for the museum.

What do we know about the visitors that come? The 'most common' age groups are children below 11 years of age; 26% are adults between 25 and 34 years of age. Of particular interest is the fact that children between 11 and 16 form only 3% of our visitors and young adults between 17 and 20 form only 11%. There is therefore some justification for regarding the Natural History Museum as a 'kids' museum, for children under 11. It appears that people come as young children, only to return when they are parents with their own children. So the vital years in education between 11 and 20 provide less than a quarter of our audience.

Approximately two-thirds of our visitors have completed their full-time education but eight out of ten have no qualifications in biology. Half of all visitors are visiting the museum for the first time and eight out of ten have not visited the museum on a previous occasion within the past year.

But in a summary of results in 1977 from our Annual Visitor Survey we found that many people set out to visit the museum with the expectation of learning something; when they left they had learnt practically nothing, or, more to the point, they gave no evidence of having learned anything, and not one that we interviewed had been stimulated to search out information for him or herself.

I think we are entitled to draw a few tentative conclusions from this. There is little evidence that we are fulfilling the Advisory Board for the Research Council's stated aim for the museum of attracting youngsters to science; and there is little sign of the university student who is supposed to make so much use of the public galleries in the museum. There would seem to be a distinct need for a new approach if we are going to fulfil the terms of the 1753 Bill.

A paper submitted to Trustees for their consideration in February 1972 initiated the new exhibition scheme. Entitled 'A Proposal for a New Approach to the Visiting Public', it starts by discussing the existing exhibition: "it is piecemeal and conceptually static, neglectful of natural processes and reflects to its loss the division of the museum into five separate departments." The 1972 paper says that the new

exhibition should deal with all forms of life, with the origin of the Earth and its life, and with the major geological processes which have affected life. It should point out areas of doubt and speculation and should complement the other national museums in London. It would have a uniquely comprehensive opportunity to display the full range of natural history. The paper suggests that the content of the new exhibition should be grouped under four headings: man, ecology, life processes and behaviour, and evolution and diversity.

There is no intention of reducing the number of organisms on display. On the contrary, the policy is to expand the exhibits illustrating diversity especially in invertebrates and plants which will be dealt with properly for the first time in the history of the museum.

My department of public services was created in January 1975 specifically to implement this new exhibition scheme. We want to make the museum an exciting place where the layman can enjoy exploring and discovering natural history. We need to attract an audience that is representative of the general public. We must recognise the need to motivate visitors.

Departmental policy gives rise to a long-term plan, which was represented by four documents written by museum scientists, members of all the departments. About 40 were involved in all and the four documents cover the four themes. The first three documents have been in the library of the museum since November 1972, the fourth, on evolution and diversity, since October 1973, and open for people to read and send in their comments. The evolution and diversity document, for example, says: "... the aim of the new exhibits on these topics should demonstrate the varied form and structure of species now existing; the historical relationships between them, and their status in the latest stage of continuing process of change on the surface of the Earth." These exhibits will contain the vast majority of the material now on show in the museum. The public will expect us to continue to provide what they have always found to be the most entertaining aspect of the museum halls of monsters. On a more profound level, we hope the visitor will be able to derive an intellectually satisfying explanation of his origins, his unity with terrestrial life, and his uniqueness and his role. The exhibits will include Recent and fossil species and will be ordered systematically by relationships.

There is a problem to be solved: the diffusion of knowledge to the general public. It is not easily solved, but by a process of trial and error it is possible to develop better solutions. Our whole approach to the operation of mounting the exhibits is based on this assumption. □

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Whither the Natural History Museum?

IN his account of the thinking behind the new exhibition schemes, Dr Miles has posed clearly for the first time a fundamental dichotomy that exists in the concept of the role of the museum. The Department of Public Services has taken an unequivocal stand that its duty is primarily to serve the general public, and more than that, not to provide mere displays of the materials housed, but to communicate ideas and concepts.

The fundamental issue was summarised succinctly at the meeting by Professor T. S. Westoll. If one sees the role of the museum as one of social engineering, then one follows one route, by implication that of Miles' current policies. But if one sees it as a unique place in which to collect together in a systematic way what is contained in the natural world then clearly the national collection as a repository, with public galleries exhibiting those materials, will develop in a very different manner. It was evident that Miles' conception took no account of the needs of students but was aimed primarily at young people with no knowledge of biology and he saw his main task as one in which he would motivate them to learn. As a body, an audience of both professional and amateur palaeontologists, zoologists and geologists was in the main unsympathetic to Miles' thesis.

By far the most disturbing aspect of the discussion which followed Miles's talk was the strong impression of a serious breakdown of communication between the Museum's Department of Public Services and the scientific staff of the Natural History Museum, such that one of the world's leading ichthyologists on the museum staff was provoked to protest publicly at the recent removal of the fish exhibits to make way for the Human Biology Exhibition. It is known that the Museum's dinosaur expert would much have preferred a completely modernised Dinosaur Gallery, emphasising the natural history of these extraordinary creatures, rather than the entirely new exhibit, largely concerned with reptile relationships, which is now being constructed in the Central Hall. The museum has announced its forthcoming publication on the new dinosaur exhibit and yet its own expert is not involved in its production. This in spite of the fact that as well as being acknowledged as a world expert, he is one of the most experienced people around at communicating his subject to young people and the general public.

Dr H. W. Ball, the Museum's Keeper of Palaeontology, has publicly stressed on numerous occasions that the museum has the responsibility to curate, conserve and display the material objects in its charge.

It has always been assumed by people outside the museum, it now transpires mistakenly, that the public galleries reflected the responsibilities of the museum's

scientific staff working in conjunction with the exhibition staff. The fact that the new exhibition scheme seems to be going ahead in spite of serious opposition from the scientific side of the museum is surely a matter of deep concern.

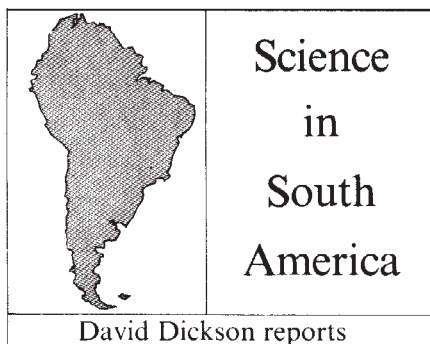
The rigid dichotomy posed by Miles between the needs of the general public on the one hand and university students and teachers on the other is assuredly false. The late lamented dinosaur gallery was a case in point. It served the needs of the general public but also those of students. A still extant example of the same thing is the fossil mammal gallery. This scholarly exhibition is filled with genuine specimens with explanatory diagrams and models accompanying them. The essence of visiting this gallery is that one can see for oneself the actual evidence on which models and restorations have been based. Visitors have the possibility of deriving their own concepts, drawing their own conclusions. This exhibit can be visited at different stages in life, from a small child, to the general adult public and to advanced students. At each level something of real value can be gained.

Once it is conceded that it is an appropriate role for a national museum to be concerned with aspects of social engineering by promoting concepts that happen to be current in the present climate of opinion, then a most dangerous precedent is set, which has sinister implications. Suddenly it becomes possible to visualise museums contributing to the indoctrination of the more inarticulate sections of the community. Just so long as natural history museums are primarily concerned with displaying the materials in their charge, there is always the possibility that the facts will shine through the prevailing dogmas.

The new scheme as epitomised in the Hall of Human Biology leads to the grave suspicion that henceforth natural history specimens will become simply three dimensional illustrations of a given narrative aimed solely at "an audience that is representative of the general public". In my opinion the museum should be nearer to scientific literature than to the media.

The Natural History Museum's new exhibition scheme raises such fundamental issues that it deserves to have the fullest possible public discussion and for this to be effective it is essential that the views of the museum's scientific staff should be fully heard, together with those of teachers and students in schools and universities both here and abroad, who are intimately concerned with the signal service that the museum has provided in the past.

We can only hope that sufficient pressure can be brought to bear to curb the activities of the Public Services Department and to ensure the survival of the museum's reputation for scholarship in its public galleries. □



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How problems in developing the Amazon region have boosted the status of environmental scientists

Brazil learns its ecological lessons—the hard way

The results of opening up the Amazon have been far from those intended: vast tracts of sterile land

SEVEN years ago Brazil's military rulers became the bogeymen of Western environmentalists when they announced that concern for the environment was a luxury of the industrialised countries which developing nations could not afford.

Today the military remains in power; but the message is very different. When President-elect Joao Baptista Figueiredo spoke recently of future development policies for the Amazon region, his references to the need for the "preservation of ecological equilibrium" to which permanent forest areas were "indispensable" could have been lifted from any environmental handbook.

The lesson has been learnt the hard way. Not primarily by listening to scientists and ecologists who, for the past 15 years, have been warning of the destructive effects of uncontrolled development on one of the world's major natural resources; but from experiencing the failure of ambitious plans to "recolonise" the region using techniques developed elsewhere and ill-suited to local conditions.

The result has been a growing awareness in official circles that the environmental impact of development programmes cannot be ignored. "In the past few years all sides have begun to see that poor environmental control can create bottlenecks in economic development," says Dr Paulo Nogueira Neto, a former biology professor who is now head of the environmental protection division of the Ministry of the Interior.

A by-product has been an increased willingness to involve environmental scientists, to whom government planners are now turning as they realise that successful development requires,

inter alia, a firm grounding in detailed knowledge of the behaviour of biological systems and their component parts.

The price of failing to respect such systems has been dramatically illustrated by the effects of the 14,000 miles of highway that have been driven through the Amazon forests like a military exercise over the past decade. The purpose of the roads has been both to open up trade with the continent's west coast, and to encourage the "recolonisation" of the region by settlers who would, it was hoped, convert the biological richness of the forest lands into valuable primary products.

Many of the results have been far from those intended. The roads themselves, disrupting streams and rivers and destroying delicate ecological balances, have produced vast tracts of sterile land. And the settlers, after a year or two of intense cultivation on land from which the trees had been removed, found agricultural yields drop sharply as nutrients were leached from the soil, and soon left to return to the urbanised south.

Thus has developed a practical awareness that, despite all its apparent robustness, the tropical forest is a fragile system in a state of precarious equilibrium; "a desert covered by trees" as it has been called by ecologist Robert Goodland. Less than a fifth of the Amazon region contains productive soil, and agricultural techniques developed in temperate zones have shown themselves frequently inappropriate to tropical conditions.

"The road was a school for us. You have to pay for everything that you do, and Brazil has paid quite a lot for its mistakes," says Dr Antonio Machado, programme director of the National Research Council's humid tropics pro-

gramme, a 3-year \$15 million project on the use of forest resources partly financed by the International Development Bank.

"But although we learnt by the wrong way, we now know much more about the soil, the forests, the water and the weather, and the effects on these of human settlements. And with this information we now have enough to start trying to exploit the forest in a rational way".

One research centre which has been brought into prominence by the new-felt need for scientific knowledge is the National Institute for Amazonian Research (INPA). Founded 20 years ago and financed by the National Research Council, the institute is set in 45 acres of deliberately-untouched secondary forest on the outskirts of Manaus, the main trading town on the Amazon river 1,600 kilometres from its mouth.

Much of INPA's work, like that of the older Goeldi Museum in Belém which is now part of the institute, continues a tradition of study of the unique biological systems of the Amazon region that stretches back through Charles Darwin to Alexander von Humboldt. And previous government antagonism to the opinions of scientists stemmed from the fact that many of the latter tended to see a direct conflict between their interests in "undisturbed" natural systems and the goals of development programmes.

Under its present director Dr Warwick Kerr, who was appointed three years ago on secondment from the University of São Paulo, attitudes have changed. Dr Kerr's outspoken views on social issues have frequently ruffled feathers in the military hierarchy, but he is committed to the belief that INPA's activities must be directed towards the well-being of the people of the Amazon region—part of a com-

promiso which he claims all research institutions have with the society that supports them.

Dr Kerr warns that at current rates of exploitation, the Amazon region's 2.7 million square kilometres of forest would disappear in less than 30 years; and that the urgent need, to which scientists must contribute, is for a pattern of controlled development that takes into account both the potential and the vulnerability of the region's resources.

Referring to the fertile *várzea* regions which receive their nutrients from annual flooding, for example, Dr Kerr says: "the floods, instead of being sources of discomfort, should be looked upon as a blessing and a source of wealth. We should learn how to cope with them, developing systems of housing, of experimentation with livestock, of conserving the forests essential to both fish and to man, and better systems of river transport".

INPA's activities reflect this commitment. There are now over 30 research scientists working at the institute—when Dr Kerr was appointed his arrival is said to have doubled the number of PhDs from one to two—and the work is divided into six divisions: biology, medical sciences, parasitology, agronomy, fish and fishing, and technology.

Each division combines both basic and applied research. In the fish section, for example, research topics range from fish biology to the life-cycle of the manatee, once a plentiful source of meat but now almost extinct through over-hunting; and the agronomy section includes both Dr Kerr's work on the genetics of bees and methods of soil management.

INPA also runs an annual programme of "intensive training" for work in the Amazon. The course, which lasts six months and currently has about 40 students, combines a study of Amazonian ecology and tropical hygiene with groundwork in research methodology (including statistics) and general laboratory techniques.

The growing concern of both local and federal development agencies for the environmental aspects of their programmes has led many to consult INPA for advice. And such interaction has helped INPA to develop a working relationship with government agencies which had initially been suspicious of its attempts to broaden the scope of its activities.

Thus for some time there has been tension between INPA and the Brazilian Agricultural and Ranching Research Bureau (EMBRAPA) over a mutual interest in agricultural research for the Amazon region. Much of this tension was removed, however, by a meeting of the staffs of the two in-

stitutions at the beginning of August, where hostility was transformed into an enthusiasm for cooperation, although with general agreement that INPA should remain responsible for the more basic aspects of the research.

A commitment to "useful" research has not been allowed to swamp more traditional interests of long-term value in the fauna and flora of the Amazon region. A major programme of theoretical ecological studies is being carried out under the direction of Dr Herbert Schubart. And a new venture, which is being organised jointly with the US-based World Wildlife Fund, involves investigating the minimum area that can be isolated as a self-contained ecosystem retaining a full range of biological and genetic diversity.

A local agency responsible for developing methods to exploit the forests resources has agreed to set aside 62 plots of land, ranging in size from one-tenth to 10,000 hectares, for the study. Before the plots are isolated and regularly thereafter for a number of years inventories of the species found on each plot will be compiled by research workers from INPA. "The preservation of the tropical rain forests and the life-forms it contains is a number one priority in our attempts to protect the biological diversity of the planet," explains Dr Thomas Lovejoy, programme scientist of the WWF, a frequent visitor to INPA, and principal investigator of the project.

Both the WWF and INPA have also been contributing to plans drawn up by the Brazilian Institute for Forestry Development (IBDF) to create a series of nature reserves — "conservation units"—in the Amazon region, which at present contains only one of Brazil's eight national parks. The areas recommended as reserves have been carefully chosen by drawing on the scientific records of the critical habitats of different species, and identifying the areas of the Amazon

where these overlap.

The government, partly under pressure from international bodies such as the United Nations Education and Cultural Organisation (Unesco), has already shown itself more willing than in the past to incorporate such proposals in its development plans. A major problem, however, is that much of the land in Brazil is privately owned, and thus outside direct government control.

"The government attitude has certainly been much better than previously. But our main problem is that although we already have eight national parks throughout Brazil covering almost two and a half million acres, less than half a million acres belongs to the government," says Dr Maria Padua, head of the IBDF team that drew up the "conservation unit" proposals.

In the Amazon region, despite attempts to stimulate the development of small-scale enterprises, most of the running has so far been made by large private enterprises. Volkswagen de Brazil, for example, has bought 300,000 acres of forest which it has burnt down for conversion into a giant cattle ranch; and near the mouth of the Amazon an elusive US billionaire, Daniel K. Ludwig, has bought a million acre estate, cut down much of the forest to grow rice and installed a \$300 million Japanese pulp mill.

Dr Kerr's wish for a controlled development that meets the needs of the Amazon people therefore takes on a special meaning. Brazil's growing ecology movement, drawing on biological and environmental arguments, favours a development strategy based on small, relatively self-sufficient communities, allowing the forest to produce a sustained yield of natural products; economic pressures however remain weighted towards large-scale enterprises primarily seeking high-level, often short-term, return on investment.

Infra-red telescope will not be ready for Jupiter mission

ONE of the things in life that even the National Aeronautics and Space Administration has been unable to speed up is the hand-finishing of large telescope mirrors. Consequently a current source of embarrassment to the agency is a last-minute delay in completing work on a 120-inch mirror to be installed in its infra-red telescope at Mauna Kea in Hawaii.

The telescope has been commissioned by NASA at a cost of \$6m, and will be used for ground-based observations of planets and other bodies in the solar system to complement data gathered

by spacecraft.

Its first task was to have been to pin-point the position and movement of "hot-spots"—thought to be gaps in the cloud which reveal the higher temperatures in the atmosphere beneath—on the surface of Jupiter. This information would have been used by the two Voyager spacecraft to direct infra-red sensing equipment and plan the sequencing of operations to gather scientific data.

The two spacecraft were launched last year on courses that take both past Jupiter, and Saturn, and Voyager

1 is due to reach Jupiter early next March. However, delays in finishing the mirror, which is being prepared in the workshops of the Kitt Peak Observatory in Arizona, mean that the telescope is not likely to be ready until the end of that month, and completion has been officially scheduled for the beginning of April.

NASA is now planning to rely on the 200-inch Mount Palomar telescope as well as an 88-inch infra-red telescope in Hawaii, to make the necessary observations both prior to and during Voyager 1's fly-past. But officials admit that the quality of the data will be inferior to that eventually expected from the 120-inch telescope.

The delays in the new mirror came first in developing new computer-based procedures for testing the quality of the mirror; and when these procedures were recently completed and the tests applied, it was found that an additional three months' work was still required.

"We are doing all we can to speed things up. But it is the kind of delicate work that does not lend itself to working in shifts; you need a complete crew that is in tune with the project and with each other", Mr Norman Cole, master optician at Kitt Peak, said last week. Mr Cole has also been responsible for the mirrors not only of the 4-metre telescopes at Kitt Peak itself and at Cerro Tololo in Chile, but also of an 84-inch infra-red telescope built by the University of Wyoming with funds from the National Science Foundation, which came into operation last year.

The team preparing the mirror has eight members, and polishers work in the final stages by removing only a few millionths of an inch at a time from selected spots. Mistakes are difficult and timeconsuming to correct: "If you go overboard in one touch, then it takes time to blend it back into the surface", explains Mr Cole.

Scientists at NASA's Jet Propulsion Laboratory in California are disappointed that the Mauna Kea telescope will not be ready for the experiments that they plan to carry out when Voyager 1 passes Jupiter, but are confident that the other two telescopes will produce adequate data, and that Mauna Kea will be ready for Voyager 2's fly-past in July.

However the delay is likely to lead to questions in Congress, which was told in 1974, when considering an initial funding request for the telescope, that "no existing telescope" was able to provide the data necessary for planning the Voyager missions, and was assured earlier this year that construction was on schedule.

Dr Bill Brunk, NASA's head of planetary astronomy, admitted last week that the situation was an "embarrassing" one for the agency; "I can only answer that we tried our best to finish it on time". **David Dickson**

Saudi Arabia invests in fishing

THE Red Sea is one of the poorest seas for fishing in the world. It is formed by a deep narrow trench making it very poor in nutrients. In the few areas where there are fish a specialised fishing industry has never been developed because of the many varieties of fish. The Saudi government, however, is trying to modernise the local fishing industry and introduce fish farming along the Red Sea coast. The scheme is being conducted by the Ministry of Agriculture and Water Resources.

In 1976, the ministry signed an agreement with the British White Fish Authority for a four year cooperation programme. So far the programme has assessed the fish resources of the Red Sea and the Persian Gulf and has studied catching methods and ways of increasing fish landings. An aerial survey has shown that there are 72 fishing communities on the Red Sea coast with a fleet of 1,225 boats. Most of these boats are traditional "dhows" which are rather inefficient. The estimated annual landing is 10,000 tons.

Any new additions to the fishing fleet must be easy for local fishermen to use and for local shipyards to maintain. The design criteria have been met by the Cornish boatbuilders Cygnus Marine who have already produced two vessels on an experimental basis. Initial indications are that these small, 10m fishing vessels will play a major role in the fishing industry of Saudi Arabia. A fleet of 25 such boats could increase the catch in the Gulf by 25%.

The Ministry of Agriculture and Water Resources also operates a research vessel, *Ibn Majid*, which has facilities for all forms of fishing. It has

discovered new fishing grounds in the south where there are more nutrients.

The modernisation of the Saudi fishing fleet, however, does not solve the Saudi fishing industry's basic problem—the lack of fish in the Red Sea and the Gulf. Consequently, the idea of fish farming has gained considerable ground. Normally, fish farming is expensive; in Saudi Arabia, however, four factors work in its favour:

- the large market for fish.
- the abundance of unpolluted sea water. Despite an increase in pollution after the re-opening of the Suez canal, the Red Sea remains one of the cleanest seas in the world.
- the abundance of cheap energy for pumping water through fish ponds.
- fish farming would transform large arid coastal plains with no other agricultural potential into food producing

areas.

The Fishery Development Laboratory in Jeddah has studied soil samples from probable locations for fish farms. A large area of the Saudi coastline is suitable for the pond/tank method of fish farming. Fish are reared and fed in fenced-off sea pens.

The farms would consist of 25m long ponds perpendicular to the line of the coast. Clean water will be pumped from beyond the reef edge to a header channel from which it will flow by gravity through the ponds before discharging into a channel which runs back to the sea. The ponds will be designed to farm three species: mullet, rabbit fish and a local favourite known as "Bouliti". Fish farming is expected to produce 829 tons of whole fish a year.

Ziauddin Sardar

Stevenson to introduce new DNA legislation in US

SENATOR Adlai Stevenson, chairman of the Senate Subcommittee on Science, Technology and Space, has announced that he intends to introduce new legislation covering research involving recombinant DNA techniques soon after Congress reassembles next January.

According to Stevenson, even though "public opposition to the research has virtually disappeared" as a result of federal actions taken so far, a number of reasons remain to make legislation advisable. These include the fact that federal support of research on

the risks of recombinant DNA techniques will be necessary to keep pace with the development of the techniques themselves; that it remains "anomalous" to restrict one class of researchers but not another merely on the basis of their location and source of funds (current guidelines now under final revision by the National Institutes of Health would apply only to NIH-funded research); and that "a programme of voluntary compliance by and monitoring of industrial research activities (as proposed in the revised guidelines) is insufficient". □

The Nobel Prizewinners 1978: Physics

A sensitive ear for the Big Bang

Michael Rowan-Robinson writes:

A MODEST and laconic letter entitled "A measurement of excess antenna temperature at 4080 Mc/s" reached the *Astrophysical Journal* on 13 May, 1965. The paper, only 600 words long, describes in a brief, almost brusque, style the first project undertaken by two young radioastronomers since their move to Bell Telephone Laboratories. Using a 20 ft horn reflector built by Bell Labs engineers for the "Echo" satellite project, they had measured the temperature of the antenna when pointing in the zenith (vertical) direction. They had found a temperature of 6.7 K (degrees Kelvin, or absolute), of which 2.3 K was due to atmospheric absorption and 0.9 K was due to Ohmic losses in the antenna. This left 3.5 ± 1.0 K unaccounted for. The two astronomers had worked very hard to eliminate noise sources in their antenna, but could not explain the excess.

Had this paper appeared in the *Bell System Technical Journal* like the other work with the horn antenna, astronomers might hardly have noticed it, as they hardly noticed Jansky's earlier discovery at Bell Labs of radio emission from the Milky Way. Penzias and Wilson did not themselves immediately recognise the significance of their discovery. However they had the good fortune to be near Princeton, where the group led by Dicke and Peebles were only too aware of the significance of a 3 K background. In early measurements with the radiometer which he invented, Dicke had noted in 1946 that the temperature of any cosmic background radiation was certainly less than 20 K. He had then turned to other experiments and to the development of the Brans-Dicke cosmology. By 1965 Dicke, in collaboration with Peebles, had independently reinvented the hot Big Bang model of Gamow, was predicting the existence of cosmic microwave background radiation with a blackbody spectrum (in seminars if not in print) and had two students, Roll and Wilkinson, building a system to measure the temperature of this background.

The plans and dreams of the Princeton group must have been rudely shattered by the news of Penzias and Wilson's discovery. The four of them wrote a beautiful paper explaining the significance of the radiation as the relic of the "primeval fireball" phase of the hot Big Bang, and outlined the physics of this radiation-dominated, opaque



Arno A. Penzias

"To Dr Arno A. Penzias and Dr Robert W. Wilson, Bell Telephone Laboratories, Holmdel, New Jersey, USA for their discovery of the cosmic microwave background radiation."



Robert W. Wilson

phase of the universe's history. This paper appears immediately preceding the one by Penzias and Wilson, with the far more dramatic title "Cosmic blackbody radiation".

Penzias and Wilson included only one very cautious remark on the significance of their 3 K background: "A possible explanation for the observe excess noise temperature is the one given by Dicke, Peebles, Roll and Wilkinson in a companion letter in this issue". Penzias had heard about the Princeton group's ideas and invited them over to Holmdel. Without that visit the only reference to the excess noise might have been the one that

appears in a paper by Penzias and Wilson on the flux from the radio source Cas A, received in its original form by *Astrophysical Journal* on 1 April, 1965: "The equivalent temperature of the antenna when pointed at the zenith is about 7 K, 2.3 K of which is due to absorption by oxygen in the atmosphere".

While it is probably inevitable that Dicke and his group would have discovered the cosmic blackbody radiation within a year or so, it was also inevitable that Bell Labs would sooner or later identify any source of background noise in the microwave region. Earlier they had, through Jansky, identified the effect of the Milky Way in the radio region. It is taking nothing away from Penzias and Wilson to say that part of the credit for their discovery must go to the thorough, professional and single-minded work of the Bell Lab scientists and engineers over the years in developing sensitive receivers and reliable antennae. As Penzias and Wilson note in their historic paper, the 3 K excess is implicit in earlier work by Bell Labs scientists with the same antenna (Degraesse, Hogg, Ohm & Scovil).

The discovery of the microwave background radiation came early in the careers of Penzias and Wilson. It was Penzias's third paper and Wilson's fourth. Subsequently they measured the temperature of the background at 21 cm and 2.6 mm and set limits on its large- and small-scale anisotropy, but the bulk of their later work has been in microwave molecular line astronomy. Penzias has exerted a very considerable influence on the development of this field, always with Wilson's brilliance with instrumentation at his right hand. In collaboration with Jefferts, also of Bell Labs, they discovered the most important interstellar molecule apart from hydrogen—carbon monoxide—and with him and other collaborators they were responsible for the discovery of microwave line emission from 6 other interstellar molecules: CN, SiO, CS, CH₃CN, OCS, and H₂S in the early 1970s. In the past 5 years their main interest has been in the relative abundances of interstellar isotopes and whether these vary from place to place in the Galaxy. They have recently designed and built a novel millimetre-wave telescope. Typically, Bell Labs insisted that the dish be built in New Jersey, rather than at some astronomically more favourable, high-altitude site, Bell Lab's interest in

the telescope is for communication with other human beings rather than with the cosmos.

One curious feature of the story of the discovery of the cosmic blackbody radiation is the ignorance of the scientific literature of all concerned. Neither the Bell Labs nor Princeton workers seem to have been aware of Gamow's idea of a hot Big Bang (*Nature* **162**, 680; 1948) or the prediction of Alpher & Herman in 1948 of a background temperature of 5 K (*Nature* **162**, 774; 1948). More forgivably they had not noticed McKellar's 1941 calculation that interstellar CN absorption line ratios implied a CN

excitation temperature of about 2.3 K (Publications of the Dominion Observatory **7**, 251).

When the Nobel committee opened the doors to astronomy with the 1974 award to Ryle and Hewish, many of us felt that it must be only a matter of time before the discovery of the cosmic blackbody radiation was similarly rewarded. It was one of the astronomical discoveries of the century, transforming cosmology into an exact science and demolishing the already shaky Steady State Theory. The blackbody nature of the spectrum is qualitatively confirmed by the most recent balloon-borne experiment (Woody & Richards,

Phys. Rev. Letters, to be published) and now interest focuses on possible distortions of the spectrum. The isotropy of the radiation both on the large and small scale is well-established apart from the small effect of the Earth's motion in space (see *Nature* **270**, 9; 1977). While we must spare a thought for Dicke and Peebles, who came so close to making this discovery, and who contributed so substantially to its interpretation, and for Jansky, who must surely have shared one of these astronomy prizes had he lived, there is no doubt that the Nobel committee have chosen deserving prize-winners. □

Fifty years of scientific achievement

David Shoenberg writes:

THE "basic inventions and discoveries in the area of low temperature physics" for which Peter Kapitza has been awarded a Nobel Prize in physics were essentially completed over 30 years ago. His many friends and admirers will, however, regard the award as a well merited and, indeed, long overdue recognition of over 50 years of scientific achievement in a much wider field and of the inspiration and guidance he has provided for many generations of physicists in England and the Soviet Union.

As a young man in his mid-twenties he was one of the enthusiastic group led by A. F. Ioffe in Leningrad who were building a school of Soviet physics in the difficult days following the Revolution and its aftermath of civil war and famine. He was already known for his originality and ingenuity in solving practical problems and he was chosen as one of a delegation sent in 1921 to establish scientific contacts with the West.

Kapitza came to Cambridge and was very keen to work under Rutherford in the Cavendish Laboratory. Legend has it that Rutherford was rather cool at first and said the Cavendish was full, to which Kapitza replied by asking what Rutherford regarded as a reasonable experimental error in his work. When told that it was usually 2 or 3%, Kapitza triumphantly pointed out that since there were 30 or so researchers, one more would be just about within the margin of error and this ingenious argument persuaded Rutherford to admit him.

During the 13 years he stayed in Cambridge he became somewhat of a legend not only for his scientific achievements but for the broad sweep of his ideas, his love of argument, his

'To Professor Piotr Leontevitch Kapitza, Institute of Physical Problems, USSR Academy of Sciences, Moscow, for his basic inventions and discoveries in the area of low temperature physics.'



Piotr Leontevitch Kapitza

enormous repertoire of anecdotes (recounted with great verve in a species of broken English which was entirely his own), his absentmindedness and above all his personal charm. At his informal weekly seminar, the Kapitza Club, as it was called, he loved to stir up discussion by asking provocative questions and often new ideas would be thrown up which later proved important.

After some early work on nuclear

physics, Kapitza took up the study of physical properties in very high magnetic fields and at very low temperatures. He developed a novel technique for obtaining fields of over 30 T by discharging a considerable energy into a coil during only 1/100 s, a time short enough to avoid much Joule heating. He liked to boast that he was probably the highest paid physicist in the world since his total work in a year lasted only a few seconds. To attain the low temperatures, he invented a new kind of helium liquefier in which the bulk of the cooling was provided by adiabatic expansion. The difficulty of lubricating the expansion engine at low temperatures was avoided by using a rapid stroke and effectively using the gas as a lubricant. Kapitza's machine was in fact the forerunner of a liquefier developed on similar principles, but for factory production, by Sam Collins at MIT. The Collins liquefier opened up the field of low temperature physics to anyone with money enough to buy a machine and so Kapitza's invention was in a sense responsible for the explosive growth after the war of low temperature physics all over the world but particularly in the United States.

In 1934, soon after the opening of the Royal Society Mond Laboratory, which had been built to house Kapitza's equipment and just as he was preparing to exploit his techniques systematically his work in Cambridge was brought to an abrupt end. During a routine visit to Russia he was unexpectedly refused permission to return to Cambridge and told that he must work in Russia instead. In spite of urgent pleas by Rutherford and diplomatic interventions at the highest level, the authorities were adamant and eventually Kapitza had to submit and

go through the wearisome process of designing and equipping a laboratory all over again. In this he was greatly helped by Rutherford, who persuaded Cambridge University to sell much of the specialised equipment (or duplicates of it) to the new Institute for Physical Problems, as it was called, and Kapitza was able to start work again early in 1937.

Soon after the Institute was operational Kapitza took up the study of the remarkable properties of helium II, the phase of liquid helium below the λ -point (2.18 K). Keesom's discovery in Leiden that it appeared to be a "super-heat-conductor" had caused great interest and in 1938 Kapitza in Moscow and Jack Allen in the Mond Laboratory in Cambridge simultaneously discovered that helium II was also a "superfluid" which could flow through narrow apertures without any driving pressure head. Eventually further experiments, particularly on the thermal effects associated with flow, led to the idea that helium II was a kind of intimate mixture of a "superfluid" which moved completely freely but carried no entropy and a "normal fluid" which did carry entropy and suffered viscous resistance to flow.

This "two fluid model", originally proposed on a semi-empirical basis by Tisza, was a little later more fundamentally established by Landau quite independently, as part of the basic quantum theory of superfluid helium he developed. [Landau was the head of the theoretical group in Kapitza's Institute and his theory proved to be seminal since it not only explained the existing experiments, but predicted several exciting new effects, which were looked for and found under Kapitza's leadership.] It was mainly for this theory that Landau was awarded a Nobel Prize in 1962.

For over 40 years the Institute for Physical Problems has under Kapitza's lively guidance continued to be in the forefront of low temperature research, particularly on superconductivity and the dynamics of electrons in metals as well as on the behaviour of liquid helium.

Kapitza himself, however, has turned to other fields. One technique to which he has made major contributions is the use of expansion turbines to liquefy air on an industrial scale in order to separate oxygen by fractional distillation. But his major activity during the last 25 years and still continuing to-day has been the use of high power microwaves to heat plasma with a view to providing the right conditions for nuclear fusion.

It is remarkable that at 84 he is still as vigorous and enthusiastic as ever. Perhaps another Nobel Prize is yet to come. □

The Nobel Prizewinners 1978: Medicine

From modest beginnings . . .



Hamilton O. Smith, Daniel Nathans and Werner Arber

‘To Dr Werner Arber of the University of Basel, Switzerland and Dr Daniel Nathans and Dr Hamilton O. Smith of Johns Hopkins University, Baltimore, Maryland, USA for the discovery of restriction enzymes and their application to the problems of molecular genetics.’

Richard Roberts writes:

THE restriction endonucleases, which have become so familiar to the molecular biologist, have finally come of age with the award of this year's Nobel Prize in Physiology and Medicine to Dr Werner Arber of the University of Basel and to Drs Daniel Nathans and Hamilton O. Smith of Johns Hopkins University. They each played a critical but separate role in drawing attention to these bacterial enzymes which dominate so much present research. Amid the proliferation of sophisticated techniques which characterise modern molecular biology it is the restriction endonucleases more than anything else which have made possible the dissection and analysis of the very large eukaryotic genomes, have allowed spectacular advances in DNA sequence analysis and have triggered the important field of recombinant DNA research. The recent major discoveries of overlapping genes and RNA-splicing were made with the assistance of restriction enzymes and it is becoming increasingly difficult to imagine how it was possible to do without them.

The first hints that restriction enzymes existed came in the early 1950s when Dr S. Luria and his colleagues reported a phenomenon they termed host-induced variation and which is now known as host controlled restriction-modification. They observed that bacteriophages grown in one strain of bacteria were often able to grow only poorly in a second strain. The few progeny emerging from the second strain, however, were able to grow

perfectly normally upon reinfection of the second strain, but now grew poorly upon the first strain. This phenomenon attracted the attention of Dr Werner Arber and by 1962, he and a colleague D. Dussoix, had conducted a series of elegant genetic experiments which provided impressive evidence concerning the nature of this "variation". Together they proposed a molecular mechanism to explain host controlled restriction-modification.

They postulated that certain bacterial strains contained an endonuclease (restriction enzyme) which was able to cleave unprotected DNA, and furthermore that those same strains must contain a strain-specific modification enzyme responsible for the protection of the cell's own DNA from the action of the restriction enzyme. Upon infection by an unprotected bacteriophage the phage DNA was destroyed by the restriction enzyme, except for some small fraction which was modified before encountering the restriction enzyme. Such modified phage were then able to grow perfectly normally upon reinfection of this same strain.

Following the detailed characterisation of the biological phenomena, both in Arber's laboratory and others, this system was analysed *in vitro*. In 1968 Arber and his colleague S. Linn, demonstrated the activity of the *Eco* B restriction enzyme and M. Meselson and R. Yuan purified a similar enzyme from *E. coli* K. It is now known that these Type I restriction enzymes recognise a specific sequence of nucleotides within a DNA molecule, but

cleave the DNA at random positions often far removed from the recognition site. Because of the random cleavage these Type I enzymes have not proved valuable as tools of the molecular biologist. Arber's studies, however, set the stage for the discovery of a second type of restriction endonuclease.

In 1970, Dr Hamilton O. Smith and his colleague K. W. Wilcox, described the outcome of experiments initially aimed at studying recombination in *Haemophilus influenzae*. The introduction to their paper states, "We made the chance discovery of what appears to be a similar enzyme (to the Eco B and K enzymes) in *Haemophilus influenzae*, strain Rd". Thus was described the isolation of the very first Type II restriction enzyme in a discovery destined to revolutionise the practice of molecular biology. In an accompanying paper, written with a colleague T. R. Kelly, it was shown that this enzyme called *Hind* II both recognised and cleaved a specific sequence of nucleotides within a DNA molecule. It thus resembled the Type I enzymes in its recognition properties, but differed significantly in its cleavage properties. It is this difference (the specific cleavage induced by the Type II restriction enzymes) which is responsible for their value to the molecular biologist. The methods used by Smith and his associates to purify *Hind* II and to characterise its recognition sequence have proved generally useful and the majority of the 150 or so restriction enzymes now known to exist have been purified and characterised in a similar way.

From our present perspective it would seem a natural and obvious experimental path to use a specific endonuclease such as *Hind* II to prepare discrete fragments of a DNA genome. However, in the late 1960s gel electrophoresis, a technique critical to current experiments did not enjoy its present popularity. Arber and Smith's experiments used sucrose density gradients or viscometric assays to characterise the products of restriction enzyme digestion, techniques which mask the exquisite specificity of the cleavage products. We must also remember that the major advances in molecular biology had been made in prokaryotic systems where a wealth of genetic back-up had been indispensable to progress. Such was not the case for the small tumor virus SV40 which had just begun to attract the attention of the molecular biologist. Already it was clear that the approaches used for the study of bacteriophages, were not so easily applied to the study of SV40.

Among those studying SV40 in these early days was Dr Daniel Nathans, who worked in the same department as

Smith. After learning of the interesting and unusual properties of *Hind* II and realising the significance of the specific cleavage he set about examining the effects of this enzyme and the enzymes from *E. coli* upon SV40 DNA. Among the techniques chosen to examine the cleavage products was polyacrylamide gel electrophoresis. The results of these studies, carried out with his colleague K. Danna, were published in 1971 and the first gel showing the *Hind* II fragments of SV40 DNA revealed the intrinsic power of the restriction enzymes to allow access to the secrets of the DNA genome. For Nathans had correctly realised that the restriction enzyme cleavage sites could serve as reference points for mapping and could thus replace the conventional genetic markers. In a continuing series of

Mary Lindley writes from Baltimore:

The excitement that spread through Johns Hopkins University last week was mixed with a hint of relief that this august institution had its very own Nobel Prize at last. Although other Nobel laureates have been associated with the university, Daniel Nathans and Hamilton O. Smith are the first incumbent members of the faculty to receive the prize.

Unlike almost all other Nobel prize-winning scientists, neither Smith nor Nathans are members of the US National Academy of Sciences. Neither was aware that their work had been nominated for the Nobel Prize, and their first response to the news, received by telephone early in the morning of 11 October, was a mixture of shock and disbelief. After the initial surprise had subsided, however, they were quick to acknowledge the contributions of the colleagues who had helped and influenced them during crucial stages.

Smith recalled how he and a graduate student, Kent Wilcox, became interested in restriction enzymes in 1968, while working on bacterial recombination. In the course of that work they incubated the bacterium *Haemophilus influenzae* with DNA of the bacterial virus P22, and were puzzled by finding that the DNA was broken up. Smith then recalled the work of Werner Arber in Switzerland, who had laid the genetic foundations for an understanding of the process whereby bacteria can destroy foreign DNA (and who now shares the Nobel Prize). And he thought also of M. Meselson's report of a restriction enzyme produced by the bacterium *Escherichia coli* K that could attack and break down foreign DNA.

Smith and Wilcox then set to work to look for such an enzyme in *Haemophilus influenzae*. By the spring

elegant experiments Dr Nathans has taken advantage of the many facets of restriction enzymes to further his studies of SV40.

As so often happens in science a rather slow and modest beginning suddenly leads to an explosive burst of interest and a rush of new knowledge. Arber's painstaking studies leading to the formulation of the molecular mechanism for host controlled restriction-modification allowed Smith to appreciate the nature of the enzyme activity he discovered in *H. influenzae*. The potential value of this kind of enzyme as a tool for research was realised by Nathans. Today it is scarcely possible to pick up a biochemistry journal without being inundated by the restriction enzyme acronyms. □

of 1969 they had found and purified it. But then Wilcox was drafted into the army, leaving Smith to continue the work alone. He progressed with much help from Bernard Weiss, working in a neighbouring laboratory, and later with the vital collaboration of Thomas J. Kelly Jr, newly graduated from Johns Hopkins University Medical School. Smith and Kelly soon identified the specific site on the DNA where the enzyme made its attack.

Meanwhile Smith had written to his friend Nathans, then working in Israel, about the interesting enzyme he had found in *Haemophilus influenzae*. At that time Nathans was interested in the relatively unexplored molecular biology of the virus SV40. He recalled that he had been wondering how he could obtain pieces of its DNA. Smith's letter provided the answer. He realised that restriction enzymes that attacked specific sites could be used to manipulate DNA in the same way that the enzymes trypsin and chymotrypsin were used already to analyse proteins.

Back in his laboratory in Baltimore, early in 1970, Nathans confirmed that restriction enzymes could attack the DNA of SV40. Then, together with colleagues K. J. Danna and G. H. Sack Jr, he set about using them to investigate the structure and function of that viral DNA. The work made rapid progress, and eight years later restriction enzymes were among the hottest properties in molecular biology. And then came the early morning phone call.

Of the contributors to this section, Michael Rowan-Robinson is at Queen Mary's College, London, David Shoenberg is at the Mond Laboratory, Cambridge, Richard Roberts is at the Cold Spring Harbor Laboratory, New York and Mary Lindley is on sabbatical from Nature at Johns Hopkins. A report on this year's prize-winner in chemistry, Peter Mitchell, follows next week.

news and views

Are deserts ecosystems?

from Peter D. Moore

THE second International Congress of Ecology (INTECOL) was held recently in Jerusalem* and the choice of venue made it inevitable that some emphasis would be placed upon desert ecosystems. Or should one refer to the deserts as ecosystems at all? N. Meir (Hebrew University, Jerusalem) introduced a controversial, if semantic, note early in the proceedings by stating that since interaction between species is of little importance in desert communities, the understanding of desert ecology involves simply the sum of autecological information about the component species. The ecosystem concept, he claimed, is of little value in such habitats. This led to the impromptu construction of a desert ecosystem model by H. T. Odum. Evidently it is still respectable in some quarters to regard deserts in ecosystem terms.

Meir's point (originally made by Darwin) about the low level of interactions between species in deserts, however, cannot be disputed. Plant density is low, mortality is largely density independent, pollination mechanisms are largely unspecialised and mycorrhizal relationships are rare. Seed predation can be very heavy on the other hand: often 90% of a plant's seeds may be eaten by rodents. The animal/plant interaction may be delicately balanced and plant species which are particularly dependent on seed production (r-strategists) may have their populations controlled by seed predators. S. R. Morton (University of California, Irvine) pointed out that there is about three times the number of granivorous mammal species in the North American deserts than in the deserts of Australia. Seed predation in Australia is still very high, however, but birds and ants frequently assume this trophic role. He suggested that this could be due to the late (Oligocene) arrival of rodents in Australia which

would have permitted the diversification of birds and ants into the available niches.

The main constraints on the flora which prevent competitive interactions developing are evidently the high temperatures and lack of water in the deserts and some accounts were given of remarkable adaptations to these extreme habitats. For example, there are the endolithic microorganisms found within porous rocks, such as sandstones and limestones. E. I. Friedmann and R. Ocampo-Friedmann (Florida State University) described such organisms from both the cold deserts of the Antarctic and the hot deserts of Sinai. The outer layer of rock, about 1 mm in thickness, is usually devoid of living matter, but its pores are often blocked by debris. The next 1 mm layer is occupied by photosynthetic algae (often with fungal associates in a primitive lichen symbiosis) or by cyanobacteria or colourless bacteria. Nitrogen is generally not fixed by these organisms, but is available from the rock or from atmospheric fixation. The blockage of pores in the outer layer effectively seals the inner system, leading to water conservation. The advantage of such a closed hydrological system is evident when one considers the data of L. Kappen and O. L. Lange (University of Würzburg) who have shown that some fruticose desert lichens, such as *Ramalina massiformis* demand relative humidities of greater than 90% before there is a measurable net gain in CO_2 . This means that only in a brief period after dawn, following nightly dew absorption, is there a net photosynthetic gain. Such a species can only achieve an annual relative growth rate of $195 \text{ mg CO}_2 \text{ g}^{-1} \text{ dry weight}$. Hiding in the rocks has its advantages.

P. J. Weisser (University of Chile, Santiago) described a rather similar site adaptation in a species of the cactus genus *Thelecephala* from the Atacama desert of Chile. This area is practically devoid of plant life except

along the coast where fog is a major source of precipitation. Here the *Thelecephala* species was found buried beneath a layer of sand 0.5–2.0 cm thick. Presumably the higher humidity of this microhabitat makes it attractive, despite the fact that the photosynthetically active radiation at a depth of 1 cm is reduced to about 1% of that at the surface.

The prevention of water loss, either by habitat specialisation or by more conventional stomate control, has its cost in terms of production. I. R. Cowan (Australian National University, Canberra) considered water use efficiency in plants in terms of the relative sensitivity of transpiration rate and assimilation rate to changes in stomatal apertures. Changing environmental conditions, such as increasing aridity may place those species at an advantage which have the capacity to vary this ratio, for example facultative crassulacean acid metabolism plants.

Such evidence concerning adaptation in desert plants demonstrates how natural selection in these environments has operated, in Darwin's words, through a "struggle with the elements". Evolution in deserts has apparently been dominated by this struggle rather than by competitive interactions between organisms.

Yet this is not the whole story, for it is among desert shrubs that one occasionally finds regularity of dispersion which betrays an underlying intraspecific competitive interaction which limits seedling recruitment. Such regular patterns have been described both in North America for *Larrea tridentata* (see Fonteyn & Mahall *Nature* **275**, 544; 1978) and in Asia for *Zygophyllum eurypterum*. Interspecific interactions are also known, as was described at the INTECOL meeting by A. Danin (Hebrew University,

* Held 10–16 September.

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Jerusalem). Some halophytic species, such as *Reaumuria hirtella* excrete salt from their leaves, thus increasing the salinity of their adjacent soils; other halophytes may have the same effect as a consequence of the shedding of salt-rich leaves. The outcome is suppression of the germination and establishment of glycophytic annuals in their vicinity.

M. M. Caldwell (Utah State University, Logan) provided one example of a situation where one desert shrub had been replaced by another. The competition was between *Ceratoides lanata* (C3 species) and *Atriplex confertifolia* (C4 species) in the Great Basin Desert of North America. Carbon from the roots of *Ceratoides* had $\delta^{13}\text{C}$ values of -22 to -25 , whereas that from *Atriplex* had values of -18 to -19 ,

which is to be expected from C3 and C4 plants respectively. But when soil organic matter carbon was examined for the layers beneath areas now occupied by *Ceratoides* it was found that upper levels had a $\delta^{13}\text{C}$ of -20 to -23 , whereas deeper layers provided values of -15 to -18 . Evidently the C3 species has invaded and replaced *Atriplex* in areas which once it dominated. The C4 system does not always come out on top.

To say that competitive interactions within or between species in deserts are unimportant is thus an oversimplification of the actual state of affairs. It may be that the adaptations which competitive selection has imposed upon desert species are subtle and must be sought at a metabolic level. □

Does Venus have an intrinsic magnetic field?

from C. T. Russell

DECEMBER on Venus promises to be very busy this year. Every 18 months the energy required to launch a probe to Venus reaches a minimum. During this year's energy minimum or, as it is more commonly called, launch opportunity, the USA and the USSR each launched two spacecraft to Venus. The two US spacecraft are scheduled to arrive on 4 December, (an orbiter) and on 9 December, (a 'bus' carrying one large and three small atmospheric probes). The USSR spacecraft are expected to arrive at Venus in late December and are generally believed to consist each of a lander with a flyby 'bus' rather than an orbiter. Thus in December, we expect that Venus will be visited by six atmospheric probes, two of which will make measurements of the Venusian surface, one bus spacecraft that will burn up in the atmosphere and two that will pass by, and an orbiter which will stay for some time making measurements to altitudes as low as 150 km above the surface.

One of the key questions to be addressed by the orbiter spacecraft is whether Venus possesses an intrinsic planetary field similar to those of the Earth and Mercury. Initial observations with Mariners 2 and 5 and Venera 4 suggested that Venus might not in fact have an intrinsic magnetic field. However, reexamination of some of these earlier data (Russell *Geophys. Res. Lett.*, **3**, 125, 1976) and more recent reports of the Venera

9 and 10 orbital magnetic measurements (Dolginov *et al. Lunar Science IX*, **256**, 1977) have provided evidence in favour of an intrinsic field. The situation is far from clear, and the recent report of plasma measurements by the Venera 9 and 10 wide-angle plasma devices (Verigin *et al. J. geophys. Res.*, **83**, 3721; 1978) does little to resolve this question. There is no doubt from the Venera 9 and 10 data that the obstacle deflecting the flow over the forward hemisphere of the planet is the ionosphere. The authors suggest a stagnation point, or effective obstacle height, of 500 km altitude. In fact it is probably even closer to the planet than this. More recent Russian radio occultation data indicate 260 km to be a more likely obstacle height. Thus any intrinsic planetary field plays only a minor role in deflecting the solar wind. With the solar wind penetrating this close to the planet, charge exchange of the solar wind with the hydrogen geocorona could be an important mechanism in decelerating the solar wind ahead of the planet as suggested by Wallis (*Nature*, **233**, 23; 1971). This mechanism would at a very minimum in the subsolar region weaken the Venus bow shock which slows down and heats the supersonic solar wind before it hits the ionosphere. The authors reject this hypothesis but show no data in the subsolar region. It is still possible that no well-defined shock transition exists in this region.

Verigin *et al.* also report on measurements in the wake region behind Venus. Here they find a plasma

magnetic tail similar in many respects to the terrestrial magnetotail in which a "magnetic field prevents plasma from filling the region behind the obstacle." However, the authors leave open the question as to whether the tail field arises from intrinsic or ionospheric sources.

Verigin *et al.* also compare the Venera data with the Soviet Mars data. In many respects the Mars and Venus plasma measurements are similar, but Mars seems to stand off the solar wind further above the ionosphere than Venus. The authors suggest that this fact indicates that the Martian obstacle to the solar wind is different from the Venusian obstacle, and that the Martian obstacle is indeed an intrinsic field. On the other hand, I find the other evidence for a Martian magnetic field less convincing than for Venus (*Geophys. Res. Lett.*, **5**, 81; 1978).

In short the question of the existence of intrinsic planetary magnetic fields for the Earth's two nearest neighbors is still very controversial. We expect that further analysis of the Venera 9 and 10 data and the new data to be acquired in December will resolve this question for Venus. However, there are no plans to my knowledge to probe the Martian magnetic field until at least 1982. At that time the American Galileo spacecraft, as presently scheduled, will whiz by at an altitude of closest approach of 275 km on its way to Jupiter. If I were polled on the eventual outcome of these investigations I would vote yes for a presently operating Venusian magnetic dynamo and no for a Martian dynamo. □

Sex pheromones in bacteria

from J. R. Saunders

THE role of sex pheromones in mating is well established in higher organisms and fungi. In contrast there is scant evidence for such chemical sex attractants in bacteria. In Gram-negative bacteria the formation of aggregates of bacteria during conjugation is well known (see for example, Achtman & Skurray in *Microbial Interactions* (Series B, Receptors and Recognition Vol 2) 233 (ed. Reissig) Chapman and Hall, London, 1977). The molecular mechanisms involved in genetic transfer during bacterial conjugation have been intensively studied but much less is known of the signalling processes which might initiate contact

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THE sea moths are a small family (Pegasidae) of marine fishes found only in the Indian and western Pacific oceans, from East Africa to Hawaii. They are enigmatic little animals encased in a hard exoskeleton and with relatively small fins except for the pectorals which are close behind the head and which open fanning out in the horizontal plane. It is these large pectoral fins, wing-like at a first glance, which gave them the name of sea moths. In life these fins are 'fluttered' and the fish moves in short bursts over the sea bed—as W. E. Gosline and V. E. Brock (*Handbook of Hawaiian fishes*, 290: 1960) observe of captive fish "this species acts . . . like a not very large butterfly". Curiously for such interesting-looking animals very little seems to be known of their natural history. Most seem to live in shallow water (down to 200 m) on sandy bottoms, presumably relying on cryptic coloration and irregular swimming action to avoid predators, although one species is described as being red in colour with blue eyes!

While their biology and life style are poorly known there is a wealth of discussion, and disagreement, over their systematic position. Recently, T. W. Pietsch (*Copeia* (3) 517; 1978) has reviewed the previous literature and on the basis of his studies of the skeleton of the sea moths has at last provided firm evidence for their classification. As Pietsch points out two main schools of opinion have existed in the past. The earliest discussions placed them with the pipefishes, sea horses, and their relatives, a stance that was general up to the early twentieth century. However, Günther (*An Introduction to the Study of Fishes*, 482–483; 1880) and

Sea moths pinned down

from a Correspondent

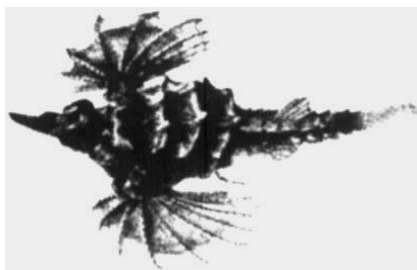


Fig. 1 *Pegasus draconis*. After Day, 1878.

others, impressed by resemblances to certain scorpaeniform fishes (gurnards, scorpion fishes, and especially the poachers) aligned them with this group. Later authors followed this lead, some more tentatively than others, but the general consensus of opinion during the present century has been that they were a distinct order, Pegasiformes, close to but distinct from the scorpaeniform stem.

As a result of an elegant study of the skeleton of *Pegasus*, Pietsch has shown that its upper jaw in particular seems to be unique amongst living fishes although similar, if not identical, to the jaw mechanism of the fossil *Ramphosus* from the Lower Eocene of Monte Bolca and Denmark. There has been general agreement that most of the structures of *Ramphosus* place it amongst the syngnathiform fishes (such as pipefishes). This suggested that *Pegasus* may well be closely allied to the pipefishes and their allies and Pietsch has found a number of significant features which support this view. In addition to the similarities in cranial osteology, the most anterior vertebrae are

elongate which is a feature typical of the syngnathiform fishes; also the gill filaments of *Pegasus* are tufted and lobe-like, not laminate as in most fishes, again a feature of the pipefishes. Pietsch argues convincingly from these and other features that the true relationships of the sea moths lie in fact with the pipefishes and their allies, not with the scorpaeniform fishes.

His further conclusions regarding the classification of these enigmatic little fishes is that they lie intermediate between the two main groups, on the one hand the sticklebacks (themselves a group with a chequered history of classification) and on the other the pipefishes and their allies. All three groups are now united in the order Gasterosteiformes. To allow for the absorption of the new group within the order Pietsch has put forward a new classification in which the sea moths represent a new superfamily. As he points out, this is tentative, as further investigation of several presumed relatives is still required.

It is mildly ironic that these little-known fishes, early examples of which mostly reached Europe as pinned specimens in Chinese insect boxes, have now been placed alongside the sticklebacks and pipefishes which are probably the most intensively studied fishes. The cynic might also point out that in this new classification the sea moths are merely returning to the position they occupied in the mid-nineteenth century. However, on account of Pietsch's careful anatomical comparisons and detailed discussion it seems that this time they will be firmly pinned down—not in insect boxes—but in between the sticklebacks and the pipefishes within the order Gasterosteiformes.

between donor and recipient cells. Although mating signals have been postulated in conjugation little convincing proof has yet been obtained for the presence of specifically 'sexual' attractants. A recent paper from D. Clewell's laboratory (Dunny, Brown & Clewell, *Proc. natn. Acad. Sci. U.S.A.* 75, 3479; 1978) now provides evidence for the existence of a sex pheromone in the Gram-positive bacterium *Streptococcus faecalis*, a normal inhabitant of the gut. Some strains of this organism possess plasmids specifying a number of characteristics including haemolysin production, bacteriocin production and antibiotic resistance. Certain of these plasmids can promote gene transfer by

a process requiring cell-to-cell contact and resembling conjugation in Gram-negative bacteria (Jacob & Hobbs 1974; *J. Bact.* 117, 360; 1974). It has been found that donor and recipient strains of *S. faecalis* form clumps in mating mixtures. Clumping has the effect of bringing donors and recipients into close contact with each other and obviously increases the probability of successful transfer of DNA during conjugation. The formation of these clumps or mating aggregates in *S. faecalis* is apparently induced by an extracellular, heat stable protein of molecular weight between 1,000 and 10,000. This protein, termed a clumping inducing agent (CIA) is produced, perhaps surprisingly, only by recipient (female) cells and not by donors (males). Furthermore, cell-free preparations of CIA will cause donor cells

to adhere even in the absence of recipients. However, CIA is not a physical agglutinating agent but rather a signal (pheromone?) which induces in donors a sequence of events resulting in cellular adhesion. Donor cells must be exposed to CIA for 30 min before clumps are formed. During this time the CIA seems to induce the synthesis of RNA and protein species which are necessary for clumping.

A further interesting property of the *S. faecalis* mating system is that recipients show a reduced ability to elicit CIA once they have acquired a self-transmissible plasmid thereby becoming male. This implies that streptococcal sex factors can regulate CIA either by switching off synthesis of the protein or by inactivating pre-existing molecules of CIA. This would seem to be reasonable in terms of biological econ-

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omy as it would be wasteful for two male strains to mate with each other.

The mode of action of CIA is itself intriguing. It will be necessary to determine the precise nature of the macromolecules induced in donors by CIA and how these could engineer the clumping response. It is possible that CIA may have an additional function in initiating the mobilisation of DNA for transfer in conjugation. The discovery of CIA in *S. faecalis* should stimulate further research to determine whether sex pheromones are a more general phenomenon amongst bacteria. □

Cosmochemistry and evolution

from A. H. Olavesen and
N. C. Wickramasinghe

AN international workshop on Cosmochemistry and the Origins of Life, organised by University College, Cardiff, was recently held at Gregynog Hall, Wales. A broad overview of the conventional ideas of chemical evolution was provided by C. Ponnampuruma (University of Maryland), who pointed to numerous laboratory experiments which showed the production of biochemical monomers from suitably energised mixtures of CH_4 , NH_3 and H_2O . Such conditions are usually postulated to have occurred on the primitive Earth. A. W. Schwartz (University of Nijmegen, Netherlands) provided further evidence for the laboratory production of biochemicals from simpler chemicals. A wide range of biochemicals could be synthesised from 70% eutectic mixtures of HCN in water. For instance all the purines were formed spontaneously in such systems, but pyrimidine synthesis required further ultraviolet-catalysed rearrangements. Phosphorylation reactions were envisaged as being derived from active phosphorylating agents arising from the interaction of apatite with certain other products of the HCN reactions. Reactions involving HCN were thought to have occurred most probably over primitive land masses whilst condensations of formaldehyde occurred in the primitive oceans. The two chemical systems are considered to have come together at the land-water interface.

H. Noda (University of Tokyo) discussed the probability of the appear-

ance of a living system from a mixture of present-day biochemical monomers. It was concluded that random molecular combinations could not have led to the emergence of life with any reasonable probability. A. G. Cairns-Smith (University of Glasgow) pointed out that it was unreasonable to expect the most primitive genetic material to involve such a complex organic polymer as a nucleic acid. He considered it more likely that the crystal structure of simpler inorganic materials, such as clay minerals, might have served as some kind of primitive gene. Clay particles might also have served to concentrate organic materials necessary for the origin of life by surface absorption processes.

M. Paecht-Horowitz (Hebrew University of Jerusalem) demonstrated that certain well-directed polypeptide synthesis could be achieved in the presence of some clay minerals. A degree of polymerisation of up to about 24 amino acids was shown to be possible in this way, and the process was thought to be related to the persistent occurrence of blocks of amino acids in present-day proteins. P. Decker (Chemical Institute, Hanover) discussed the role of autocatalytic systems in the early development of biochemistry. Feedback loops in such systems could achieve steady-state concentrations of certain biochemicals without the necessary participation of enzyme catalysts.

J. T. Wimpenny (University College, Cardiff) traced the evolution of microbes from anaerobic prokaryotes to eukaryotic structures. Evolutionary pressures are thought to have forced primitive prokaryotes to develop increasingly sophisticated systems for the utilisation and conservation of essential ingredients. He stated that further biochemical evolution was effectively predetermined at the stage at which the first eukaryotic systems emerged.

The question of the site for the origin of life pervaded the entire meeting. Most participants preferred a terrestrial origin, but a radically different proposal by F. Hoyle and N. C. Wickramasinghe (University College, Cardiff) was the subject of much discussion. Hoyle and Wickramasinghe argued that the set of biochemical monomers necessary for the origin of life is produced in mass flows from stars and is abundant in interstellar molecular clouds. Such biochemicals were mopped up into comets when the Solar System formed and high aqueous concentrations of these materials are maintained for long periods in cometary interiors where they propose that life began. Hoyle and Wickramasinghe suggested that comets still continue to shed living systems onto the Earth in the form of bacteria

and viruses. They then discussed an analysis of influenza attack rates in boarding schools (during the 1978 epidemic) which they claim provides evidence against normal explanations for the spread of this virus.

P. M. Solomon (State University of New York, Stony Brook) reviewed astronomical data relating to galactic molecular clouds. Over 40 molecular species have been detected so far and include HCN and formaldehyde which are also the most ubiquitous of the simpler organic molecules in the Galaxy. It is of interest that laboratory synthesis of biochemicals have mainly involved these two cosmically abundant species.

W. M. Irvine (University of Massachusetts) discussed radioastronomical observations of molecules in comets. Molecules detected so far include HCN, CH_3CN , H_2O and several radicals and ions. There are several radio lines in comets which are as yet unidentified. S. Yabushita (Kyoto University, Japan) discussed theories of cometary origin. K. Nandy and D. H. Morgan (Royal Observatory, Edinburgh) reviewed astronomical observations relating to an ultraviolet interstellar absorption feature centred on $\lambda=2200 \text{ \AA}$. The most likely contributors to this band are thought to be small graphite spheres of radii less than $200 \text{ \AA}$. N. Nakagawa and A. Sakata (University of Electro-Communication, Tokyo) considered the role of organic molecules with conjugated double bonds in producing the $\lambda 2200 \text{ \AA}$ band. They also discussed mechanisms for the formation of such molecules by grain surface reactions in interstellar space, and described laboratory experiments which synthesised such molecules as HC_3N , HC_5N , HC_7N and HC_9N from HCN. □

European geophysics

from Geoffrey M. Brown

OF all the branches of science which attempt to operate under omnibus umbrellas, possibly the most nebulous is geophysics. The spectrum of interest extends from what is curiously referred to as 'solid earth' to the outermost extremities of the terrestrial environment. It is evident that in any assembly of geophysicists in conference there will be no uniting theme. The recent meeting of the European Geophysical Society¹ was no exception. If one had to select a single phrase to summarise the conclusions from the wide coverage of subjects it would be to emphasise progress in techniques. Repeatedly there were references to recent advances in measuring techniques and instrumentation, the development of

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new techniques, the application of numerical techniques, and the refinement of analytical techniques.

In the symposium on active plate boundary regions of the North Atlantic, results of the long-range side-scan sonar GLORIA were presented which confirmed the importance of this technique for tectonic studies of such boundaries. New syntheses of the seafloor spreading history of the North Atlantic have been undertaken, and a major land/sea refraction experiment has shown that in the top few tens of kilometres beneath Iceland normal upper mantle velocities are absent. Partial melting is suggested by an increase in Poisson's ratio with depth.

Thanks to major improvements in palaeomagnetic and rock magnetism analysis techniques, increasing detail is now available on the relationship between successive phases of deformation and metamorphism, and on estimates of parameters such as age and temperature. The general character of the palaeomagnetic record is now so well established that claims to discover new 'anomalies', whether in general palaeomagnetism or magnetostratigraphy, must pass rigorous standards of analysis before they can be considered seriously.

In fluid dynamics, which spans the atmosphere, oceans, mantle and core, much progress has been made by combining field observations, laboratory experiments and numerical analyses. For example, in thermal convection some of the patterns (rolls, hexagons, knots, skewed varicose instabilities) identified in the laboratory, are now recognised (though not yet fully understood) in clouds in the atmosphere and even in small boulder formations in Arctic regions. The effects of condensation on mountain lee waves can now be modelled, whilst on a larger scale this is evidence that the global circulation may be quantised into a number of stable and unstable states, transition being effected by small-scale perturbations. Transition probability is a major problem.

'External geophysicists' were served by three symposia. In one, concerned with the aeronomy and dynamics of the lower ionosphere, the main message was the importance in the height region 70–90 km, of the influence of the neutral atmosphere on the ionised atmosphere. The mechanisms which control this ion-neutral coupling are being intensively studied. In general, the rate of production of ionisation is largely determined by the transport of atmospheric minor constituents, such as nitric oxide, while the rate of loss

of ionisation is strongly dependent on the extent of the water cluster-ion regime which, in its turn, is controlled by the temperature of the atmosphere. Knowledge has significantly advanced through the extension and improvement of both ground-based and rocket techniques. Particular attention was given to results from the Western Europe Winter Anomaly Campaign of 1975–76, which have demonstrated that anomalously high radio wave absorption in winter arises from both increased production and decreased loss of ionisation in the D region. Greatly improved digital telemetry in rocket experiments reveals a fine structure in both the ionisation and neutral gas density in the lower ionosphere. As another example of progress in techniques VHF radar using very short pulses integrated over a second has been used to detect very thin vertically moving layers in the height range 60–75 km.

The other two atmospheric symposia were concerned with different aspects of the acceleration of charged particles in the Earth's environment. The mechanisms which impart the necessary energy to the particles responsible for the aurora have long been elusive. In the symposium on auroral acceleration processes, experimental auroral data covering a range of parameters were compared with theoretical predictions, computer simulations and laboratory studies of plasma acceleration mechanisms. The role of electric fields parallel to magnetic field lines within an Earth's radius is being actively studied. The injection of tracer beams of electrons and ions promises to be an important new technique in the experimental measurement of such fields. Valuable new insights are expected to come from more detailed experimental study of particle distributions; there is need for clarification of the association between accelerated particles and plasma waves. The consensus was that acceleration by quasi-static electric fields is probably the most viable model to date, although it is clear that no single theory is currently capable of accounting for all observations.

In the symposium on the bow shock, consideration was given to the acceleration of charged particles in space in the collisionless region ahead of the magnetosphere where wave-particle interactions transform the cool, supersonic solar wind in the interplanetary medium into hotter, lower speed plasma in the magnetosheath. Very recent measurements aboard the first two ISEE (International Sun-Earth Explorer) satellites, only a few hundred kilometres apart, were presented which enable temporal variations to be resolved from spatial

variations for the first time. These show the presence of ions, sometimes suggestive of beams with rather well-defined angular distributions and with energies up to a few keV, believed to be accelerated by wave-particle interactions at the bow shock. They also show that the thickness of the shock may vary by a considerable factor. The detailed ISEE observations now available in space will remove some of the ambiguities of single spacecraft measurements and should lead to renewed theoretical efforts to understand the intricate physics of collisionless shocks in plasmas.

The EGS is a young society which seeks to bring together geophysicists of all types through its annual meetings. Already detailed plans are laid for the sixth meeting in September 1979 in Vienna. □

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Liposomes in the therapy of lysosomal storage diseases

from Gregory Gregoriadis

THE first lysosomal storage disease was identified by Hers in 1963, who found that deficiency in lysosomal X-glucosidase resulting in glycogen accumulation was the underlying cause of the clinical manifestations of Pompe's disease (glycogen storage disease type II). Other lysosomal storage diseases (LSDs) were subsequently discovered which involve the total or partial absence of various hydrolytic enzymes. Initial attempts at enzyme replacement therapy by the direct administration of the missing enzyme isolated from foreign and human sources were disappointing (reviewed by Desnick *et al. Physiol. Rev.* **56**, 57; 1976). Patients set up immunological reactions to the foreign enzymes and some enzymes even fail to enter afflicted cells. Enzyme is also wasted through premature inactivation or loss to irrelevant areas (see *Enzyme Therapy in Lysosomal Storage Diseases* (eds Tager, Hooghwinkel & Daems) North Holland Publishing Company, Amsterdam, Oxford, 1974).

Some years ago liposomes were proposed as suitable enzyme carriers (for a recent review see Finkelstein & Weissmann *J. Lip. Res.* **19**, 289; 1978).

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*Held at the Université Louis Pasteur, 29 August–1 September.

Biochemical and morphological observations showed that, *in vivo*, liposomes could transport agents into the lysosomes of cells (mainly those of the reticuloendothelial system and the hepatic parenchymal cells) by endocytosis. Liposomes protect their enzyme contents from circulating antibodies and prevent them from attacking normal blood components. Work with model lysosomal storage 'diseases' suggested that enzymes transported in this way into lysosomes could degrade stored materials, and recently fibroblasts from a cystinotic patient were partially depleted of lysosomal cystine after exposure to liposome-entrapped cysteamine.

Reynolds *et al.* (page 754, this issue of *Nature*) now present a method for evaluating the liposome approach to enzyme replacement therapy in a naturally occurring LSD, namely feline GM₁ gangliosidosis, which is characterised by a deficiency in β -galactosidase. Skin fibroblasts from cats with GM₁ gangliosidosis were initially pulse-labelled with radioactive galactose (galactose-labelled glycopeptides in the lysosomes of diseased cells are not catabolised over a period of days). Exposure of galactose-labelled cells to liposomes containing β -galactosidase from feline liver is followed by the catabolism of the radiolabelled glycopeptides as a result of the concomitant uptake of the enzyme, apparently through endocytosis of its liposomal carrier. In this and other cases free enzyme fails to enter the cells.

Clearly this is an attractive way to measure the efficacy of a given liposomal enzyme preparation within target cells and could be easily applied in a variety of LSDs. However, degradation of pulse-labelled substrate, obviously concerning newly synthesised materials, may not be characteristic of enzyme action on all accumulated substrate in all intracellular areas. Indeed, it is possible that lysosomes which are either aged or excessively loaded may not be able to fuse with the endocytic vacuoles transporting the liposomes or with young secondary lysosomes which have already incorporated the enzyme. In addition, much of the substrate may be in a physical state resistant to enzyme activity. Therefore, experiments are now needed to show that what Reynolds *et al.* achieved with pulse-labelled fibroblasts can also be achieved with cells at the centre of a given LSD (for example, fixed macrophages from adult Gaucher's disease patients storing glucocerebroside, cardiac or skeletal muscle cells from glycogen storage disease type II patients) by measuring total stored material and examining cellular morphology and function before and after exposure to enzymes.

Extrapolation of results from *in vitro*

experiments to the overall efficacy of the system *in vivo* could, however, be meaningless. In dealing with animals one should consider difficulties related to the journey of the carrier to areas in need of treatment. Liposomes must retain the enzyme while in the circulation and must therefore be designed appropriately. In several LSDs involving tissues other than liver and spleen, the carrier must cross membranes to reach and enter diseased cells (entrance into nerve cells seems improbable at present) and control of its size and surface characteristics will be needed (for a review see Gregoriadis *Nature* **265**, 407; 1977). Reduction in size will also ensure less interference by the liver and spleen, although the chances are that these tissues will themselves be enzyme deficient and participation in uptake is unlikely to be detrimental. Possible toxicity of liposomal lipid components and increased immunogenicity of administered enzymes promoted by the carrier must also be taken into account (discussed by various contributors, *Ann. N.Y. Acad. Sci.* **343**: 1978).

Assuming that enzymes reach target cells and degrade much of the stored material, will this then lead to the organ functioning normally? In adult Gaucher's disease for instance, liver and spleen grow enormously with con-

comitant development of fibrous tissue and other secondary morphological as well as metabolic disturbances, and in several lipidoses nerve cells are probably irreversibly damaged. Our experience (Belchetz *et al.*, *Lancet* **i**, 116; 1977) with an adult Gaucher's disease patient being treated for over 30 months with liposomes containing glucocerebroside: β -glucosidase isolated from human placenta, provides evidence that the enzyme is acting in the liver, as the transient pain in that area after each injection is proportional to the amount of enzyme given. Significantly, the patient's health has stopped deteriorating since treatment began and there is some reduction in abdominal pain due to liver pressure as well as some improvement of reticuloendothelial system function. In the only other case of LSD treatment with liposomes some reduction in liver size was also reported. Unfortunately, however, the patient (an infant with glyco-genesis type II injected with fungal amyloglucosidase) died before any valid conclusions could be drawn (Tyrrell *et al. Brit. Med. J.* **2**, 88; 1976). A major difficulty in the follow-up of LSD patients is our inability to measure progress in serial biopsies as there are gross differences in the substrate content and morphology of various parts of the tissue. □

Radiation research in Scandinavia

from R. B. Cundall

A RECENT conference* in Denmark covered most of the topical and debatable aspects of radiation science, in addition to what now appear to be well established and steadily progressing areas, such as electron trapping, excited states, and free radical reactions. The conference was attended by delegates mainly from Scandinavia, and served particularly well in the attention which was directed to controversial and timely topics. These were the disposal of nuclear waste, industrial applications of high energy radiation, and radiation effects on DNA.

Legislation recently introduced in Sweden on the disposal of spent fuel and wastes from nuclear power generation has served to concentrate much effort and thought on this subject. Papers by F. Lofveberg (Nuclear Fuel Safety Project, Sweden), A. Lund (Nykoping, Sweden), J. A. C. Marples (AERE, Harwell, UK) drew attention to the nature and properties of vitrified waste and the thought-provoking prob-

lems of storage facilities which may be used for periods of more than 10⁶ years. The possibilities of leaching, temperature effects and washing which could be significant have been examined but the results indicate that the radiation and physical stability of glass waste is of no concern. Presentations on transport of radioactive isotopes through different types of terrain also provided reassurance. This type of work is important for the safe exploitation of nuclear power, especially since the time scale of possible hazards is outside previous human experience and could involve larger quantities of dangerous material than is generally appreciated, although so far the radiation experts' findings are encouraging.

Opposing views are even more firmly established concerning possible industrial applications of ionising radiation to chemical synthesis and modification of physical properties, especially those of polymers. J. Silverman (University of Maryland), a well-known exponent of radiation technology, spoke with enthusiasm and probably surprised the delegates with economic statistics showing possible annual turnover

*6th Conference of the Nordic Society for Radiation Research and Technology, Hirtshals, Denmark, 28-31 August 1978.

"SUDDENLY, pear blossom on every tree/As though the wind of Spring came upon us last night." With these words so welcome to a people that has just passed a long and dreary Winter in science (see *Nature*, **274**, 834; 1978), a new Chinese journal, *Ziran Zazhi*, greets the world.

Ziran Zazhi means simply 'Nature Magazine'. It is a monthly of about 60 pages and its first issue came out in May. Its aims are: to get better acquainted with Nature, to remould Nature and to exact greater freedom from Nature. Its editorial policy is to "let scientific truth develop and grow through contention". It covers both physical and biological sciences and is very much like this Journal in having as its main content Articles, Research Letters and Nature Information. In addition, it has two more popular sections, Explorations in Nature and Riddles in Nature.

The Research Letters at the moment account for only 10% of the pages, but they are given pride of the place. Compared with the Letters of this Journal, they are somewhat shorter, just as technical, and cover as wide a spectrum, except perhaps for a slight bias toward particle physics and systems analysis in the issues so far published. Arguably the most original Letter so far is a report of some experimental results on the physical basis of the traditional *yunqi* therapy—the curing of disorders through the passing of *qi* (pneuma) from doctor to patient without bodily contact. Though not as well-known as acupuncture, this form of therapy has its believers and followers among

Nature: Chinese style

from T. Kiang

the Chinese people. It goes something like this: the doctor does a sort of breathing exercise; a few minutes later, he has a feeling of congestion (stage 1) which may last a couple of minutes; he then has a feeling of release (stage 2) lasting another couple of minutes. The traditional 'theory' is that, during stage 2, the doctor's *qi* is emitted through certain specific points called *xuwei* and enters the patient's body through some other specific *xuwei*, effecting the desired cure. What the experimenters found was that, during stage 2 but not during stage 1, the far infrared radiation at 1.2 cm from the active *xuwei* of the doctor showed a slow (down to 0.05 Hz) modulation of large amplitudes (up to 80%). They also found abnormalities in body electricity near the *xuwei* during both stages. Furthermore, from chemical analysis of tissues from a number of corpses, they found that the tissues at the *xuwei* points had a marked excess of nickel. These findings have all the hallmarks of pioneer work and their preliminary character makes the Research Letter their perfect vehicle.

The Articles form the mainstay of *ZZ*. Each covers in depth a certain topic—Mössbauer spectrometry, the red tide and laser chemistry have been covered amongst others — and is written by an acknowledged expert in the field. In regard to the degree of

technicality, the Articles of *ZZ* resemble more their counterparts in *Endeavour* than those in this Journal.

Nature Information consists of extracts of articles from other current scientific periodicals, notably, *New Scientist*, *Science News*, *Science* and *Physics Today*. Here, as with Articles and Research Letters, astronomy and astrophysics have their due share, but these are even more prominent in the Explorations in Nature section. In one form or another, readers have been informed of recent findings made in China and abroad on the origin of the Solar System, black holes, gamma-ray bursts, neutron stars, rings of Uranus and quasi-stellar objects that are at the same time double radio sources. For many of the Chinese investigations, the full-length papers are now available in English in the translation journal *Chinese Astronomy*.

The Riddles in Nature which have appeared so far have dealt with the life-span of single cells, magnetic monopoles and the μ -particles. I, as a professional astronomer, found the first most intriguing—which the editors of the multidisciplinary *Ziran Zazhi* may feel gratified to know.

The appearance of *Ziran Zazhi* is a heartening event. Just how heartening can perhaps be appreciated only by the Chinese scientific community. It is one of the signs that the country has begun her 'New Long March' towards modernisation through science and technology. □

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reaching billions of dollars in the USA alone. Two hundred electron accelerators and 60 industrial ^{60}Co units are already in use throughout the world. It is clear that academic workers in the radiation science area are ill informed on the industrial possibilities; for example, well publicised applications to the synthesis of detergents seem to have failed whereas the radiation-induced crosslinking of rubber for tyres has apparently been very successfully applied by the Goodyear Company in the USA. Discussion revealed caution and open scepticism from many delegates about the current state of such technology, although specific and restricted areas of application, sterilisation for example, are undoubtedly extremely useful. Industrial application depends crucially on detailed investiga-

tions and these were exemplified by papers on polyethylene/ SiO_2 composites, polystyrene sterilisation, and electrical insulation. J. Rabani's (Hebrew University, Jerusalem) interesting paper on solar energy exploitation was more of hope and problem definition than achievement. Photoelectron transfer effects which are probable mechanisms for achieving the formation of H_2 and O_2 from solar irradiation of water are likely to be enhanced by polyelectrolytes, ion exchangers and micelles. The use of polyelectrolytes seems to offer many significant advantages since they inhibit back electron transfer reactions and help fulfil the requirements of energy storage.

The interest in cellular and sub-cellular radiobiology is largely centred on the effects of radiation on DNA, and the effect of oxygen was the main theme of the presentation. The difficulties of experimentation in this clinically important field were highlighted by the

paper of B. M. Cullen and J. W. Boag (Hammersmith Hospital & Institute of Cancer Research, Sutton, UK) which showed how errors of technique can provide misleading results. Photoelectrons formed in higher yields when argon was used instead of nitrogen as deoxygenating atmosphere caused reductions in cell survival.

The still incomplete knowledge on reactions of the biochemically significant O_2^-/HO_2 system was also examined. The paper by G. Czapski (Hebrew University, Jerusalem) showed the increasing involvement of physical chemists in problems of radiation biology. The interdisciplinary approach is proving seminal, the previously accepted so-called Haber-Weiss reaction is discounted but an alternative explanation remains to be found.

No enzyme has attracted the radiation scientist more than superoxide dismutase. The reasons are its selectivity towards the O_2^-/HO_2 system and

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its convenient properties for study by pulse radiolysis. E. Lengfelder (Strahlen-biologisches Institut der Universität, Munich) showed how several synthetic metal-containing complexes also display marked superoxide dismutase activity.

As appropriate for a meeting in Scandinavia significant 'state of the art' papers on DNA by Graslund and G. Ahnström (Wallenberg Laboratory, Stockholm) and positronium (O. Mogensen, Riso Laboratory, Denmark) were presented. The effect of radiation on DNA has been and remains the most important aspect of radiobiology and it is essential that effects of individual free radical reactions, strand breakage, chemical modification and biological effects are examined and correlated. Ahnström emphasised that effects on repair processes could be crucial to radiosensitivity. Radiation damage to DNA prevents enzymic cleavage of oligonucleotides, and suggests a new method for studying radiation effects in DNA (D. Schulte-Frohlinde and C. von Sonntag, Max-Planck-Institute, Mulheim).

The successful application of resonance Raman spectroscopy to pulse radiolysis was a notable achievement of the Riso group (R. Wilbrandt, N. H. Jensen and P. Pagsberg) and details of the structure of $(SCN)_2^-$ have been determined by its use. The powerful technique of pulse radiolysis is considerably improved by transient detection methods which allow better structural identification. □

NATURE

A hundred years ago

IN a lately-issued number of the *Proceedings of the American Philosophical Society*, Prof. Cope has given an account of the collection of fishes made by Prof. Orton at various points on the head-waters of the Amazon. Species of the genera *Belone* and *Tetrodon*—characteristically marine forms—are here recorded as having been obtained in these districts 2,500 miles from the mouth of the river—thus showing how far marine animals will penetrate into, and become ultimately acclimatised in, fresh waters.

THE existence of the true heath plant in North America was for a long time considered very doubtful, and its detection in New England some years ago is a matter of much interest. The published localities hitherto are Newfoundland, Nova Scotia, and Massachusetts, but, according to the *Bulletin* of the Torrey Botanical Club, Dr Hexamer, of Newcastle, has lately found a few plants of it near Egg Harbour, New Jersey.

From *Nature* 18, 24 October, 682; 1878.

Producing more food

from W. S. Wilson

THE inexorable increase in the world's population without a commensurate increase in food supplies has prompted a re-examination of production methods, post-harvest care of produce and even the types of food consumed. These considerations were well to the fore at the XXth International Horticultural Congress*. The wide range of scientific disciplines involved focused on fruit, vegetables, ornamental and amenity plants and post-harvest horticulture. The last was a new feature of these 4-yearly gatherings and reflects the growing concern for quality produce in developed countries and the need to overcome scarcity in many developing countries, including India where per capita consumption of vegetables is only one-third of that recommended by dieticians.

Nowadays vegetables and fruits are survival foods in some countries and health-giving foods in all others. Vegetables provide more carbohydrate, vitamins and minerals per unit area of land than other type of crop. Some, such as the soya bean, are an inexpensive source of protein. A spectacular way of intensifying vegetable and fruit production in some countries was given by E. C. Quisumbing (University of the Philippines) when he described the growth of coconuts under which were growing bananas, under which were grown pineapples beneath which were grown sweet potatoes, all on the same piece of land at the same time. This arrangement resulted in quadrupling the potential yield of the land and increasing the income of the small farmers.

Apart from management aids to increasing production and improvement of produce quality, advances in fundamental knowledge and technological applications which make handling and mechanical harvesting easier, were reported. In the temperate fruit section, the emphasis was on the adjustment of tree density by breeding or hormonal treatment to render the fruit easier to harvest mechanically. This trend is particularly advanced in Australia and New Zealand, countries with small labour forces. Plant breeders have introduced dwarfing characteristics enabling more trees to be grown more productively per hectare. Another method for increasing yield of apple cultivars, described by A. I. Campbell (Long Ashton Research Station, Bristol) was the exposure of dormant scions to gamma irradiation under water to form stable mutants. Physiologists have been able to control growth characteristics

by hormonal regulation. Thus ethylene can be used to increase flowering, offset biennial bearing, prevent pre-harvest fruit drop and advance or delay fruit maturity.

The most important advances in vegetables have also been related to systems methods of production. W. L. Sims (University of California, Davis) outlined a sophisticated system for tomato production involving an integrated breeding programme, uniformly flat seedbeds, simultaneous ripening and a single harvest operation. This system is appropriate to California but other systems applicable to other regions can be devised. For less ambitious objectives crop increases can be achieved by deeper loosening of non-compacted soil or by hormonal treatment of crops to achieve greater lateral proliferation of roots to explore compacted soil. Allied with these measures is a rapid method of forecasting fertiliser requirement such as that described by D. J. Greenwood (National Vegetable Research Station, Wellesbourne, Warwick). This involves assessing inorganic nutrient ions in plant sap with test strips and in the soil filtrate with reagents prepared in tablet form. A notable achievement in vegetable breeding is the production of high protein potatoes (over 10% protein) for use as animal feed in Australia.

The main concern of the section on ornamental plants was to devise the best way of educating the community to appreciate plants, gardens and landscape. For over 300 years botanic gardens have been preoccupied with taxonomy and amenity horticulture. In the future, these institutions and horticultural societies should be more concerned to inject greater diversity into horticulture by, for example, introducing plants from the southern hemisphere into northern countries.

In the post-harvest section a paper by M. A. Perring (East Malling Research Station) on mineral and physiological disorder in apples explained how the physical and chemical composition of apples is influenced by soil type and management, environment and nutrition. In this connection, bitter pit in apples caused by calcium deficiency can be alleviated by dipping the fruits in a calcium chloride solution. Preservation of condition in easily-perishable vegetables can be achieved by covering the crop with low cost polyurethane foam in cool stores.

Australia provided an excellent venue for the Congress, offering a wide range of wild flora alongside a diverse horticulture in which science and technology are complementary. □

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review article

The biochemistry of complement

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Current biochemical studies of the complement system are illustrated by description of the activation of complement by the classical pathway after interaction with antibody aggregates. This is described in terms of the structures of the components involved, their assembly and the mechanism of activation.

IN the blood of vertebrates several activation systems have been recognised which are essential for the survival of the animal. These include blood clotting, clot lysis, the renin-angiotensin and kinin systems and complement; all are dependent, at least in part, on the sequential activation of a series of proteolytic zymogens¹.

Complement is the most complex of these systems and it is of interest for several reasons. First, as recognised 80 yr ago², the complement system is responsible for the destruction and elimination of foreign material from the body, notably bacteria and viruses. This usually occurs after the interaction of antibodies with the foreign organisms or substances, and complement is thus the effector mechanism in the immune defence against infection. Complement may also be activated by complex polysaccharides³ such as yeast or bacterial cell walls before reaction with antibody and hence it can form an immediate defence against infection before the development of immunity⁴. Second, receptors for activated complement proteins, such as the third component C3 are present on lymphocytes, macrophages and some other cells⁵⁻⁷, and there is evidence, as yet incomplete, that the lymphocyte C3 receptors may play an important part in the interaction of the different classes of lymphocytes or their precursor cells⁸. The interaction of lymphocytes such as the B and T cells is necessary for the occurrence of the immune response and it is possible that complement may play a central part in the immune response as well as being the major effector mechanism. Third, it has been found that several complement proteins are coded for by structural genes which map into the major histocompatibility locus in man and in mice⁹. The biological significance of these findings is uncertain, but it has been suggested¹⁰ that it may imply that some complement proteins have a role as surface proteins, as all the other proteins coded for by this gene complex apparently do.

Not surprisingly, therefore, interest in the complement system has increased greatly, and the progress in isolating and characterising the various components has led to attempts to determine their structure and the biochemical mechanisms of the activation system. It is highly complex and includes about 20 serum proteins, most of which are part of the activation sequence, but control proteins are also included; these inhibit different steps or inactivate some of the components. The biological importance of the system has been emphasised by the finding that it is probably present in invertebrates and has been conserved throughout evolution¹¹ to such an extent that some components are interchangeable between species as distant as frog and guinea pig¹². The complexity of the system may be due to the multiple role of some components as suggested above, but also,

as with the other activation systems of the blood, it is important that they are activated rapidly when needed but never activated accidentally. A cascade system with each step able to be inhibited offers a system of rapid activation coupled to a series of fail-safe controls.

There are several features of complement which are most unusual. For example, C1q, the component which interacts with aggregated antibody, has a unique structure which is half globular and half collagen-like¹³. Also, several of the proteases involved in the complement system are formed only after activation of the protein possessing the catalytic site and association of this protein with one or more other proteins which thus determine the specificity of the activated enzymatic site^{14,15}. No attempt will be made to review the whole complement system, but only a few of the more uncommon features will be described, of the structure and activation of the early components of complement after interaction with aggregated antibody.

Activation of the complement system

Activation of the complement system can take place by two distinct pathways, the classical and the alternative pathways: here we shall concentrate on the classical pathway. Both pathways have the same terminal components, C5 to C9, which are directly responsible for membrane damage. The difference between them lies in their early-acting components and thus in the manner by which they are activated by immune complexes or other factors. The sequence of events in the activation of the early components of the classical pathway is shown in Fig. 1. When C1 is bound to antibody aggregates it becomes an active protease, C1^s, and catalyses the activation of C4 and C2 which together form another protease C4² (ref. 14). This enzyme is also known as C3 convertase because it activates C3, some of

Fig. 1 Activation of the classical pathway by antibody-antigen (Ab-Ag) aggregates.

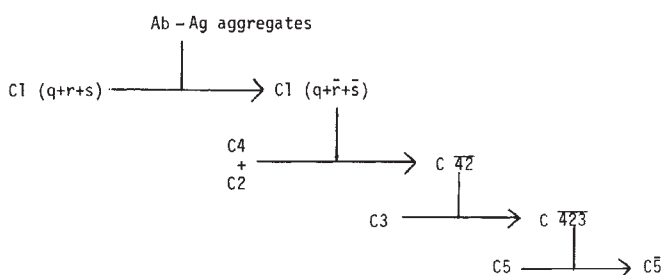


Table 1 Early acting components of the classical pathway

Component	Molecular weights of					Enzymatic site present in activated form	Approximate serum concentration (mg l ⁻¹)
	Major unactivated form			Activated form			
	Intact molecule	Chains*		Intact molecule	Chains*		
C1q	410,000†	18	6A 24,000	Unchanged		—	150
			6B 23,000				
			6C 22,000				
C1r	83,000‡		1	83,000	56,000	+	50
					27,000		
C1s	83,000		1	83,000	56,000	+	50
					27,000		
C2	110,000		1	110,000	70,000		
					30,000		
C3	180,000	2	α 105,000	170,000	α' 95,000	+	15
			β 75,000		β 75,000	—	1,200
C4	206,000	3	α 93,000	200,000	α' 87,000	—	400
			β 78,000		β 78,000		
			γ 33,000		γ 33,000		
C5	180,000	2	α 105,000	170,000	α' 95,000	—	80
			β 75,000		β 75,000		

* Chains obtained after reduction and alkylation of disulphide bonds.

† C1q is composed of nine subunits, each subunit consisting of two disulphide-linked chains (see text and ref. 13).

‡ C1r is present as a dimer of two identical, noncovalently linked chains in serum.

which associates with C3 convertase to form C423; which in turn activates C5. Some properties of components C1 to C5 are shown in Table 1. In the alternative pathway of activation, which can be started when polysaccharides are added to serum without interaction with antibody, other components lead to the formation of a different form of both the C3 and C5 convertase enzymes¹⁶. The cleavage of C5 is considered to be the last enzymatic step in the activation of complement by either pathway. The final event is the formation of the C5b6789 complex, apparently by self assembly¹⁷ in the process of which part of the complex becomes inserted into cell membranes^{18,19}, thus causing the membrane lesions seen in electron microscopy studies^{20,21}.

Structure and properties of the first component of complement

C1 occurs in serum as a weak association of C1q with a tetrameric complex of C1r₂-Ca²⁺-C1s₂ which is formed in the

presence of Ca²⁺ (refs 22, 23). Interaction of C1 with aggregated antibody is through C1q (ref. 24) which binds in the absence of C1r₂-Ca²⁺-C1s₂ to the second constant domain (N terminal half of the Fc) of IgG (refs 25–30). The C1r₂-C1s₂ complex binds to C1q on the antibody through C1r, as C1r alone, but not C1s, interacts with antibody bound C1q (refs 31, 32).

C1q is a 400,000 molecular weight protein^{33–35} formed from 18 peptide chains 6A, 6B and 6C, each of about 200 amino acids and containing 12, 8 and 4% CHO respectively³⁵. Amino acid sequence analysis showed that these three types of chain are very similar and each has about 80 residues of typical collagen-like sequences starting close to the N terminal end^{36,37}. The N terminal sequence of the B chain (Fig. 2) between residues 6 and 89 has the repeating triplet Gly-X-Y, where the residue in the Y position is often hydroxyproline or hydroxylysine. The A and C chains have similar collagen-like sequences between residues 9 and 89 and 3 and 89, respectively. (Numbering of residues is based on B chain sequence, Fig. 2.) The repeating triplet sequences in each chain are, however, not quite continuous. In chain B, alanine not glycine occurs at position 9; in chain A

Fig. 2 N-terminal amino acid sequence of the chain of human C1q. The sequences are given in the single letter code: A,Ala; C,Cys; D,Asp; E,Glu; F,Phe; G,Gly; H,His; I,Ile; K,Lys; L,Leu; M,Met; N,Asn; P,Pro; Q,Gln; R,Arg; S,Ser; T,Thr; V,Val. The numbering is based on the B-chain sequence. A gap has been made between positions 38 and 39 in the B and C chain sequences to allow for the 'extra' threonine in the A-chain sequence. A triplet gap has been made between residues 42–44 in the A and C chain sequences, as this seems to give the optimum alignment for maximum homology. The sequences shown are from refs 36 and 37 except for residues C41 to C97 which are unpublished data (K.B.M.R.). Each chain is approximately 200 residues long. * Denotes a hydroxylated residue.

	1	10	20	30	40	50	60
A-chain	E D L C R A P D G K K G E A G R P G R R G R P G L K G E Q G E P G A P G I R T G I Q - - - G L K G D Q G E P G P S G N P G						
B-chain	E L S C T G P P A I P G I P G I P G T P G P D G Q P G T P G I K G E K G L P - G L A G D H G E F G E K G D P G I P G D P G						
C-chain	N T G C Y G I P G M P G L P G A P G K D G Y D G L P G P P G E P G I P A I K - G I R - - - G P P G Q K G E P G L P G H K G						
	61	70	80	90			
A-chain	K V G Y P G P S G P L G A R G I K G I K G T P G S P G N I K D Q P R P A F						
B-chain	K V G P K G P M G P K G P P G A P G A P G P K G E S G D Y K A T Q K I A F						
C-chain	K D G P N G P P G M P G V P G P M G I P G E P G E E G R Y K Q K F Q S V F						

threonine is inserted in between positions 38 and 39 and alanine replaces glycine at position 36 in the C chain. However, with the exception of these small changes at the beginning and in the middle, there is a continuous sequence in all three chains of which the similarity to collagen is increased by the presence of glucosylgalactosyl disaccharide substitutions on the hydroxylysine residues^{33,38}. The sequences of the remaining 110 noncollagenous residues in each chain, which are known, are very similar and each has one intrachain disulphide bond. Collagen fibrils are formed from three peptide chains each in a minor helix, which associates noncovalently to form a major helix with well characterised dimensions³⁹. This makes it possible that the 18 chains of C1q are associated through their collagen-like sections to give a hexameric structure of six fibrils with six globular heads formed from the C-terminal halves of each chain. Such an arrangement would be consistent with the remarkable structure of C1q as seen in electron microscopy, showing each molecule to resemble a bunch of six tulips with the lower half of the stems held together and branching in the upper half to give six separate stems each of which is joined to a globular head⁴⁰⁻⁴².

C1q, when reduced and electrophoresed on polyacrylamide gels containing sodium dodecyl sulphate, shows the three expected polypeptide chains A, B and C in equal amounts which, though of similar size (23,000 MW), separate probably because of their differing carbohydrate content³⁵. However, if electrophoresed before reduction, two subunits can be seen which again, though of similar molecular weight (46,000–48,000), separate clearly on the acrylamide gels. The apparently larger subunit contains the A and B chains and the apparently smaller subunit only the C chain^{13,34}. That is, they correspond to an A-B dimer and a C-C dimer and yields show that there must be six AB and three CC dimers¹³. The association of these nine subunits to yield the hexameric structure seen in the electron-microscopy studies may be achieved in the way described in Fig. 3. A triple helix could be formed between the collagen region of one of a pair of C chains and the collagen regions of an AB dimer; in this manner three pairs of triple helices would be formed. Then the association of these three pairs, again noncovalently, would give the complete hexameric structure, each of the globular heads being composed of the C-terminal 110 amino acids of an A, a B and a C chain (Fig. 3)¹³. The breaks in the Gly-X-Y sequence mentioned above and occurring at positions 39 and 36 in the A and C chains, respectively, could account for the bend in the collagen-like fibrils seen midway along the stems of the molecule, as a true triple helix cannot be formed in this area of the molecule because of the prerequisite for glycine as every third residue³⁹. Measurement of the dimensions of the molecule on the electron microscope picture and calculation from the known lengths of the Gly-X-Y triplet sequence in collagen agree well¹³. It seems likely, therefore, that the postulated structure is correct.

C1r and C1s are very similar proteins, each a single polypeptide chain of about 83,000 molecular weight differing only in that C1r dimerises whereas C1s remains as a monomer in solution irrespective of the presence of Ca^{2+} (refs 43–45). Together they form a tetrameric complex in the presence of Ca^{2+} , as in serum. On activation, they are both hydrolysed to give two disulphide-linked peptide chains of 56,000 and 27,000 molecular weight, but they remain in a tetrameric complex. C1r and C1s are very similar in amino acid content and also in sequence as far as this is known, and it is considered likely that one arose from the other by gene duplication. Both activated subcomponents are typical serum esterase proteases inhibited by diisopropyl fluorophosphate^{46–48} and both have a characteristic N-terminal sequence present in the 27,000 molecular weight chain (which is the chain containing the catalytic site), showing obvious homology with other serine esterase proteins⁴⁵. C1r and C1s differ markedly in enzyme specificity, however, and C1s is the only known substrate for C1r. C1s, but not C1r, will activate C2 and C4, and C1s will also hydrolyse several peptide substrates, showing a plasmin-like specificity⁴⁵.

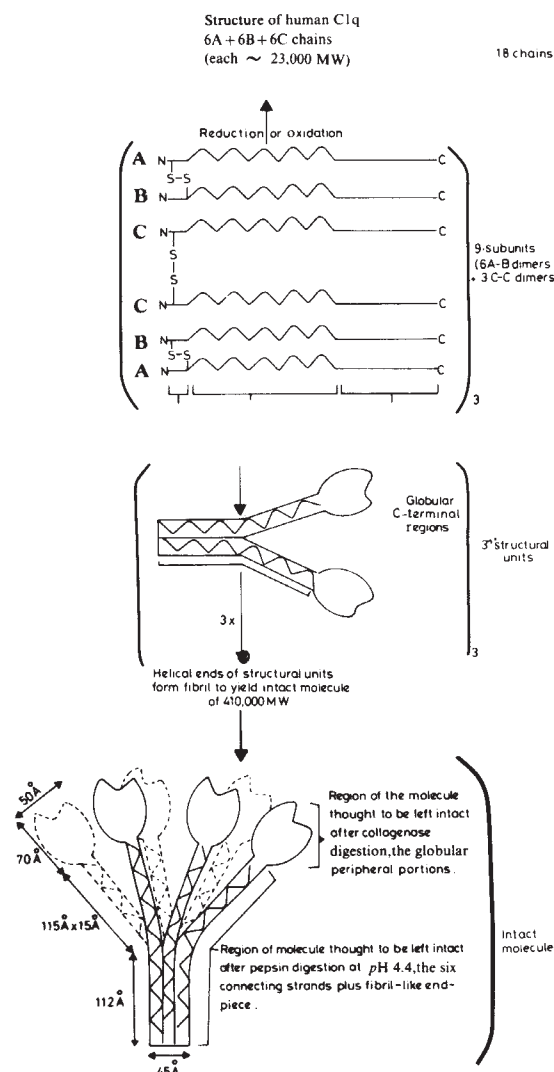


Fig. 3 Proposed peptide chain structure of C1q. The dimensions given are averages of those of Shelton *et al.*⁴¹ as estimated from electron microscopy studies. ~, Proposed triple helix sections, that is, collagen-like regions of molecule. The following comparison can be made. Length of collagen-like fibre + fibril-like endpiece = $115 + 112 = 227$ Å. Length of triple helix proposed from sequence studies = $80 \times 2.9 = 232$ Å.

Structure and properties of components C2 to C5

C4, a major complement component (400 mg per l serum) has three disulphide-linked peptide chains of about 90,000, 75,000 and 30,000 (refs 49, 50), although recent evidence suggests that it may be synthesised as a single peptide chain of 200,000 molecular weight⁵¹. Some of this precursor molecule, which is very similar to C4 as judged by antigenic specificity, but which has no haemolytic activity, also seems to be present in serum⁵². The Ss protein identified first as a polymorphic protein in mouse serum which is coded for by a gene in the major histocompatibility complex⁵³, has now been identified as C4 (refs 54–57). Human C4 has also been shown to be coded for by a gene which maps into this complex on chromosome 6 (ref. 58).

C2 has been one of the most difficult components to isolate, as it is present at only 15–20 mg per l of serum and is exceptionally vulnerable to proteolysis during isolation. Isolation has now been achieved^{59,60}, and in confirmation of earlier work^{61,62} it was found that C2 is a single-chain protein of about 100,000 molecular weight which is split on activation to two chains, not disulphide-linked, of 70,000 and 30,000. The activated protein may be a protease, possibly of a serine esterase type, though not

readily inactivated by diisopropyl fluorophosphate and, surprisingly, with the catalytic site in the larger chain⁶³. Proteolytic activity is only apparent when C2 forms a complex with C4 and then its specificity seems to be restricted to hydrolysis of C3; hence the term C3 convertase¹⁴. C2 is also polymorphic and is coded by a gene mapping into major histocompatibility complex in humans⁶⁴.

C3 (refs 65–67) and C5 (ref. 68) are proteins of about 180,000 molecular weight, each containing two chains of about 105,000 and 75,000 molecular weight. Such structural evidence as is available⁶⁹ suggests that C3 and C5 are so similar that, as with C1r and C1s, they probably arose by gene duplication. On activation, both lose a peptide (C3a and C5a) of about 10,000 molecular weight from the longer peptide chain. In each case the small peptide has anaphylactic activity^{70,71}. Only C5a has chemotactic activity⁷². C3, however, is present in much higher concentrations in serum, 1–1.5 g l⁻¹, and is equivalent in concentration to the sum of all the other complement proteins. It seems that C3, as C4, may be synthesised as a single chain precursor molecule⁷³.

Assembly of the component by addition of antibody-antigen aggregates to serum

The mode of assembly of these serum proteins on aggregated antibody and the mechanism of their subsequent activation are far from clear, but several features are beginning to be understood. The antigen seems to have no specific role and whole complement is activated equally well either when antibody is precipitated by addition of a soluble antigen such as ovalbumin, or when antibody-coated red cells are added to serum⁷⁴. Quantitatively, complement consumption per 1,000 molecules of antibody added is 1.5–2 times higher for antibody-coated cells than for antibody aggregates. However, this difference is small and may simply be because antibody molecules spread on a cell surface are more accessible than those in aggregates.

The binding of the first component to the antibody is very efficient (>95% in antibody excess) and activation is complete⁷⁴. C4 is rapidly activated by C1 but only a small fraction (<10%)^{74–76} is bound to aggregates or antibody-coated cells, the rest becoming inactivated in the serum (Fig. 4). Binding of C4 to antibody-coated cells is 10–15 times more efficient than to antibody aggregates, but the number of C4 molecules which become effective as judged by their capacity to form C3 convertase when C2 is in excess is only about twice as great⁷⁴, which is comparable to the difference in C1 binding. This suggests that most of the C4 binds to the cell surface rather than to the antibodies, and little of this is able to form C42 and continue the complement sequence. However, although it has been shown¹⁴ that C4 can bind to red cells in the absence of antibody, it can also apparently cause lysis on the addition of C2 and C1s followed by the later components of complement. The C4 on the cell surface can therefore take part in the activation sequence, presumably by binding C2 and forming C3 convertase. In this experiment, C2 would be formed in solution close to the cell-bound C4. When the antibody is on the cell surface the C1 complex containing C1s would be bound to the Fc part of the antibody and hence the C2 activated at a distance from the cell-bound C4. C2 is rapidly inactivated in serum and in these conditions is likely to be able to interact only with C4 molecules bound on the antibody molecule carrying the C1, or with immediately neighbouring C4 molecules. This could also explain why C4 bound to antibody aggregates is so much more effective than C4 bound to antibody-coated cells.

The activation of C2 is also very inefficient, but quantitative data on the number of activated molecules able to bind and form C42 complexes have not been obtained. A speculative scheme of the assembly of the early components is shown in Fig. 4 and illustrates the binding of C1q to the C_H2 domain of the antibody. The C1r₂-Ca²⁺-C1s₂ complex binds to the collagen tails of C1q and becomes activated⁷⁷. C4 and C2 in solution are activated by C1 and some of the C4 molecules bind either to antibody

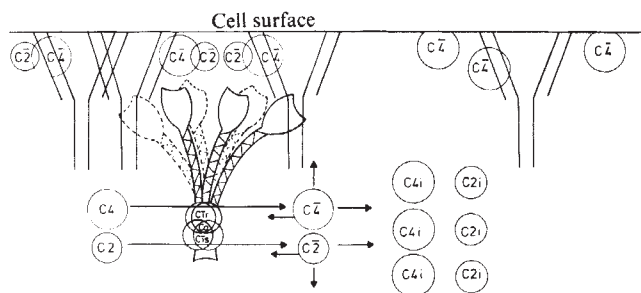


Fig. 4 Suggested assembly of the early components of the classical pathway of complement on antibody bound to a cell surface. The heads of the C1q molecule bind to the C_H2 domain of the antibody and the C1r₂-Ca²⁺-C1s complex to the collagenous tails of C1q through C1r. The activated C1s formed splits C4 and C2. Most C4 and C2 is inactivated in the serum but some C4 binds either to the Fab part of the antibody or to the cell surface. C2 binds to adjacent C4 molecules and forms C3 convertase, but is inactivated before reaching more distant C4 molecules which are ineffective in the haemolytic sequence.

molecules or to the cell surface. C2 molecules which are inactivated rapidly form a C42 complex only with C4 close to the bound C1 complex. The evidence that C4 binds to the Fab portion of the antibody is qualitative⁷⁸ or indirect⁷⁴ and hence is only tentative.

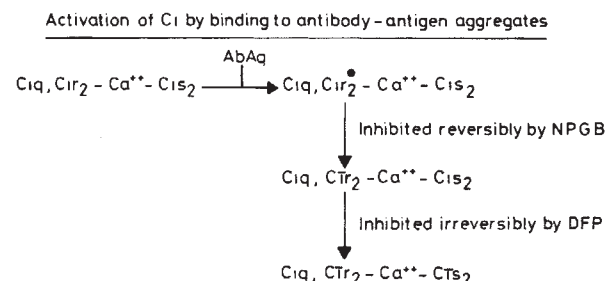
The C3 is split by the C42, and some associates to give C423 which changes its specificity from C3 activation to C5 activation⁷⁹; this is the last proteolytic step in the activation of complement. The activated C5 interacts with C6, C7, C8 and C9 to give the complex responsible for cell lysis and there is evidence that this can bind and lyse cells other than those coated with antibody and responsible for the early activation steps^{80,81}.

Mechanism of activation of the first component of complement

Of the biochemistry of the activation sequence certain mechanisms are clear. For example, in C1 bound or in solution, it is C1s which splits C4 and C2. C1q has no known catalytic activity and no covalent bonds are broken or formed when it interacts with aggregated antibody. C1r will not hydrolyse C4 or C2 in solution nor when bound to antibody aggregates as part of the C1 complex.

Similarly, C1s is not autoactivated when bound or in solution, but it is activated by C1r. It is not clear how C1r is activated²³. Although it is difficult to prepare C1r which is entirely stable at 37 °C and neutral pH, repeated addition of proteolytic inhibitors such as diisopropyl fluorophosphate (iPr₂P-F) makes possible the isolation of preparations which are stable over several hours in these conditions. Activation of C1r when bound as C1 onto

Fig. 5 When the tetrameric complex C1r₂-Ca²⁺-C1s₂ interacts with C1q bound to aggregated antibody, the conformation of C1r is changed, exposing a proteolytic site (C1r^{*}). It is this site in the unsplit peptide chain which catalyses the hydrolysis of a peptide bond and the conversion of C1r^{*} to C1r. C1r activates C1s to C1s. The active site of C1r^{*} is distinguished from that of C1r in that it reacts reversibly not irreversibly with nitrophenyl guanidine benzoate (NPGb) and it is not inhibited by diisopropyl phosphate fluoridate (DFP).



antibody aggregates occurs in 5–10 min, so satisfactory controls can be obtained³¹. It is then found that although C1q and C1r will bind firmly to antibody aggregates in the presence of Ca^{2+} , no activation occurs. C1s in the complex is essential for C1r activation but C1s or C1s inactivated with $\text{iPr}_2\text{P-F}$ ($\text{iPr}_2\text{P-C1s}$) can replace C1s. As $\text{iPr}_2\text{P-C1s}$ is effective in facilitating the activation of C1r, C1s can have no proteolytic role in the activation. Presumably, therefore the binding of the $\text{C1r}_2\text{-Ca}^{4+}\text{-C1s}_2$ complex is necessary because it determines the conformation of C1r. In the presence of 10 mM $\text{iPr}_2\text{P-F}$, C1r is still activated but C1s is not. As both C1r and C1s are inactivated readily by $\text{iPr}_2\text{P-F}$ this agrees with the evidence that C1r activates C1s but suggests that C1r will not activate C1r in the complex, as is found in solution³¹.

Activation of C1r and C1s in the complex is inhibited if 1 mM nitrophenyl-guanidino-benzoate is present, but the inhibition is reversed on removal of this compound. Nitrophenyl-guanidino-benzoate will inhibit C1r and C1s irreversibly and hence again it is unlikely that C1r activates C1r (ref. 31). However, the kinetics of C1r activation show a sigmoidal curve suggesting autocatalysis. The most likely explanation of these results (Fig. 5) is that the binding of $\text{C1r}_2\text{-Ca}^{2+}\text{-C1s}$ to the C1q on the antibody causes the C1r to take up a conformation which exposes a catalytic site in the unsplit C1r molecule³¹. This catalyses hydrolysis of C1r to C1r which in turn activates C1s.

Activation of components C4, C2, C3 and C5

C1s then hydrolyses C4 with loss of a 6,000 molecular weight peptide chain from the α chain and also splits the C2 peptide chain into 70,000 (C2a) and 30,000 (C2b) molecular weight chains⁸². The activated C4 binds either to the Fab portion of the antibody or to the cell surface and interacts with such activated C2 molecules as reach it before they are inactivated. Inactivation of C2 seems to be continuous in terms both of loss of ability to form C42 and in loss of C3 convertase activity after formation. When C2 interacts with C4 covalently attached to Sepharose and is then treated with C1s, the 70,000 molecular weight fraction, C2a, dissociates leaving C2b still bound. It has been suggested that the interaction with C4 is through the C2b part of the molecule and loss (50% in 10 min) of C3 convertase activity may be due to the dissociation of the C2a which probably contains the catalytic site, from the complex⁶⁰. Characterisation of the catalytic site and explanation as to how interaction with C4 determines its specificity will be necessary for an understanding of the mechanism of these reactions.

The subsequent step of the formation of C5 convertase by interaction of activated C3 with C42 to give C423 is even less well understood, but investigation was made possible by the finding that treatment of human C2 with weak iodine solution produced a 10–20-fold increase in its stability and activity in forming C3 convertase⁸³. Centrifugation in a sucrose density gradient of C42 using iodine-treated C2 and C3 showed the presence of two peaks of C5 convertase activity of 14–16 S and 9–10 S and a peak of C3 convertase activity at 11.3 S (ref. 84). It was suggested that the heavy C5 convertase activity could be explained by the presence of an equimolar C423 complex and the lighter by an overlap of the C42 and C3 peaks. If this is correct, noncovalent binding of C42 and C3 is not essential for C5 convertase activity, but much more detailed investigation will be necessary to establish the nature of this enzymatically active complex.

This is the last proteolytic step in complement activation and no catalytic activity has been found in activated C5 or in the lytic complex it forms with C6, C7, C8 and C9. Of the four proteases of the classical system, C1r, C1s, C42 and C423, the first two are clearly of the serine esterase type and the two enzyme complexes, where the catalytic site is believed to be in C2, may also be of this type. However, it is unusual in that it is relatively resistant to diisopropyl fluorophosphate and, more surprisingly, the active site seems to be in the 70,000 molecular weight chain⁶³, whereas in all other serine esterase type proteases it is in chains of 20,000–30,000 molecular weight. All these proteases

of the classical pathway are active in solution but are probably bound to antibody or cell surfaces *in vivo*. This is likely to be an essential feature, as plasma and other body fluids contain high concentrations of proteolytic inhibitors and activation must depend on the local excess of protease over inhibitors.

There are many finely balanced features of this highly complex system, the ramifications of which are far from clear, and the unravelling of the mechanisms of activation and control seem likely to throw new light on an animal's immune defence against infection.

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articles

High energy neutrino astronomy

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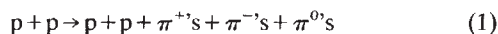
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The possibility of doing point source neutrino astronomy is discussed. Probable sources include galactic nuclei, Seyferts, quasars, radio galaxies, pulsars and supernovae.

THE possibility of building a very large deep underwater muon and neutrino detector (DUMAND)¹, which would look at the interactions of neutrinos produced by cosmic rays hitting the atmosphere has been discussed. New design ideas mean that this detector may be able to resolve the direction of origin of the neutrinos to better than 1 deg² which opens up enormous possibilities for point source neutrino astronomy some of which are considered here. These may not need to wait for DUMAND but may be realisable with existing detectors of the type being developed by Lande². For most of our discussion, however, we will use proposed parameters for DUMAND; such that a flux from a point source of order 10² eV cm⁻² s in neutrinos more energetic than 3 × 10¹¹ eV constitutes a detectable signal for a 10⁹ ton detector in one year of viewing time. (This flux produces 10 counts per yr per 10⁹ tons.) Signals as small as 10 eV s⁻¹ cm⁻² may prove to be detectable due to solid angle resolution and proper identification of background and effective detector masses larger than 10⁹ tons. The 10⁻² eV flux level is not large by astrophysical standards; the brightest non-thermal sources (such as pulsars and active galactic nuclei) have fluxes far greater than this, so if they can somehow put even a small fraction of their total luminosity in the form of high energy neutrinos, they are in principle detectable.

Any system which has high energy proton collisions will produce neutrinos due to pion and kaon production and decay in such collisions.

The production of high energy neutrinos in astrophysical systems thus entails first the production of high energy protons, followed by their degradation through pion and kaon producing collisions against other protons^{3,4}. The most important astrophysical reaction is



While the neutral mesons decay into γ rays, the charged pions eventually decay into three neutrinos and an electron (or positron) by the standard decay sequences. The cross section for proton collisions is roughly 40 mb, and is only weakly dependent on the collision. Thus protons are stopped over a path length of about 30 g cm⁻². The leading pion may contain a significant fraction of the collision energy, thus a few neutrinos may carry away a large fraction of the collision energy³.

Pions can also be produced in proton-photon collisions when the photon has an energy >600 MeV in the frame of the proton.

The cross section for this reaction depends on the collision energy and at maximum is

$$\sigma_{p\gamma} \approx 2 \times 10^{-28} \text{ cm}^2 \quad (2)$$

Diffuse sources

High energy galactic cosmic rays hitting the upper atmosphere will produce high energy neutrinos. These are the prime neutrinos which the high energy physicist might use for directly probing the nature of the weak interaction, and searching for intermediate vector bosons and Higgs particles. This diffuse atmospheric flux will be higher for those neutrinos coming from the horizon than from overhead due to the relative (atmospheric) decay length. In addition, at high energies this flux will drop off with energy by an additional 1/E due to time dilation on the decay of the π 's and k 's preventing neutrino production. The average intensity of this background in the energy range of interest, 10¹²–10¹⁴ eV, is <0.3 counts deg⁻² yr⁻¹. These considerations indicate that this atmospheric background should be easily distinguished from neutrino fluxes of extraterrestrial origin. (Direct neutrino production⁵ will not have the addition 1/E drop off but it is down $\sim 10^{-4}$ relative to π and k production.)

Berezinsky and Zatsepin⁴ and Margolis *et al.*⁴ have noted that the galactic cosmic rays interacting with the interstellar matter will produce a background which is marginally detectable and is peaked towards the galactic centre. Here, as in all astrophysical sources, all of the π 's and k 's decay so the ν -spectral index is essentially the same as that of the high energy protons with no additional 1/E time dilation effect.

Other galaxies will similarly contribute to the diffuse ν -background^{3,4}; however, if the other galaxies have the same galactic cosmic ray intensity and spectral shape as ours, then ours will dominate the diffuse ν -background. On the other hand, it is reasonable to expect that galaxies were more energetic in the past and thus produced more ν 's. Berezinsky and Zatsepin⁴ mentioned the possibility of a very early 'bright phase' of galaxies to produce very large numbers of ν 's. These ν 's will, of course, be redshifted, but they may, nevertheless, dominate the diffuse background. Thus, a measurement of the diffuse background alone would enable us to make some discrimination between a bright early phase for galaxies and the situation where early galaxies are similar to our present one.

Point sources

For the astronomer, the most appealing aspect of a large neutrino detector, such as DUMAND, is the ability to resolve

individual point sources. As mentioned earlier, a variety of non-thermal systems would be detectable if they generate a significant fraction of their luminosity in high energy neutrinos. To do so, a system must, (1) produce fast particles efficiently; and, (2) contain enough target material near the source of the fast protons to stop a significant fraction of them. Many of the most exciting objects in the sky may meet these criteria. Table 1 lists several astrophysical situations in which both these conditions may be met.

Supernovae

Standard cosmic ray models assume that a typical supernova puts out 6×10^{50} erg in relativistic cosmic rays. With the spectrum proportional to $E^{-2.5}$. The neutrino counting rate in a 10^9 ton detector is then⁶

$$R \approx 10^{-3} n / D^2 \text{ d}^{-1} \quad (3)$$

where D is the distance in kpc to the supernova remnant and n is the ambient density in atoms cm^{-3} of the medium in which the remnant is situated. Regardless of the detailed mechanism by which the cosmic rays are accelerated in either the remnant or the shock itself, the basic fact remains that if the supernova occurs in a dense cloud where η may be as large as 10^3 , it would then be a detectable point source of high energy neutrinos. Because supernovae are presumably rapidly evolving massive stars, it is reasonable to assume that many go off while still in the immediate vicinity of the cloud out of which the presupernova star formed. It is also likely that a presupernova star will undergo significant mass loss and hence create its own circumstellar cloud.

A supernova in a dense environment would also be detectable by γ -ray satellites in the energy range $E_\gamma < 1$ GeV. Indeed, coincidences have been reported⁷ between unidentified γ -ray sources detected by the Cos B satellite and radio emitting supernova remnants. The signal detected by Cos B, if assumed to be the low energy end of an $E_\gamma^{-2.5}$ γ -ray spectrum originating from π_0 decay, would yield 10 – 10^2 $\text{eV cm}^{-2} \text{ s}^{-1}$ in 10^{12} eV γ -rays, and a comparable flux in high energy neutrinos.

Pulsars

Pulsars accelerate high energy protons⁹ which if they hit something will produce neutrinos. Berezhinsky⁸ has pointed out that young pulsars, blanketed by a supernova remnant less than a year old could be bright sources of high energy neutrinos. However, their behaviour at such early times is highly uncertain. Despite these uncertainties, it is reasonable to believe that a pulsar in a binary system could be a source of high energy neutrinos for the first few thousand years of its existence¹⁰. The stellar wind of the companion could have a column density as high as 1 – 10 g cm^{-2} , its corona could provide further target material and an accretion cloud could conceivably surround the pulsar. If the companion is a red giant, its envelope could block

nearly half the high energy protons emitted by the pulsar. A pulsar emitting (roughly the luminosity of the Crab pulsar) $10^{37} \text{ erg s}^{-1}$ in high energy particles ($E_p > 10^{13} \text{ eV}$) at a distance of D kpc produces

$$R = \eta D^{-2} 10^2 \text{ counts d}^{-1} \quad (4)$$

where η is the fraction of the luminosity converted to high energy neutrinos by the target material near the pulsar. A typical value of η might be between 10^{-3} and ~ 1 .

Helfand and Tademaru¹¹ have speculated, on observational grounds, that a significant fraction of all pulsars form in binaries. Stepanian *et al.*¹² have detected a flux in high energy γ rays ($E_\gamma > 3 \times 10^{12} \text{ eV}$) of about $60 \text{ eV cm}^{-2} \text{ s}^{-1}$ from Cyg X3, which is generally held to be a (heavily blanketed) pulsar in a binary system. A comparable flux in high energy neutrinos would be detectable with a 10^9 ton detector in a year or two of viewing time. Any process—such as Colgate's¹³ shock mechanism—which produces high energy particles promptly during the initial supernova explosion will produce a prompt burst of neutrinos if the exploding star has a binary companion. For example, in the type I supernova model where a white dwarf accretes beyond the Chandrasekhar limit from a red giant companion, the explosion will, of course, occur in the vicinity of that companion. The narrow pulse detected at Earth would contain a maximum of

$$N = \eta 10^8 D^{-2} (U/10^{48} \text{ erg}) \text{ counts}$$

where U is the energy output of relativistic particles, and η is the fraction of them stopped by the binary companion and surrounding material. A typical value here for η might be as high as 0.5 .

Galactic nuclei

The most potent sources of high energy neutrinos may be active galactic nuclei¹⁴, such as those of radio galaxies, Seyferts and possibly quasars. Such nuclei are capable of luminosities as high as 10^{43} – $10^{47} \text{ erg s}^{-1}$. As the radiation seems to be of non-thermal origin, it is believed that the power outputs in high energy particles take on similar values. As these high energy particles would be produced within the innermost regions of the nucleus, which have column densities well in excess of a typical galactic disk, the high energy protons produced there might be expected to be stopped by nuclear collisions before escaping the nucleus. (Consider, for example, that cosmic rays in our own galactic disk go through a few g cm^{-2} of matter before escaping. Were the column density of the disk a factor of 30 higher, the dominant loss mechanism would be nuclear collisions.) Moreover, it can be argued¹⁵ that the accretion column surrounding a collapsed object in the nucleus may have column densities of 10^2 – 10^3 g cm^{-2} , which would be sufficient to even stop the γ rays, but the ν 's would still easily escape.

Thus, one could reasonably expect that such active nuclei put out a significant fraction ($\approx 10\%$) of their entire luminosity in the form of high energy neutrinos. The nearby peculiar galaxy M82 (often thought to be an exploding galaxy because of its optical appearance) could give a neutrino count rate in excess of 1 per day if, indeed, there are nonthermal processes occurring in its nucleus. Other nearby active nuclei, such as Cen A and M87 could give count rates of the order 10 per year. Grindlay *et al.*¹⁵ reported a high energy ($E_\gamma > 3 \times 10^{11} \text{ eV}$) γ -ray flux of the order of $50 \text{ eV cm}^{-2} \text{ s}^{-1}$ from Cen A. A comparable flux in high energy neutrinos would be detectable within a year of viewing time. (Remember that the atmospheric background per deg in a 10^9 ton detector is $\leq 0.5 \text{ counts yr}^{-1}$ thus as few as 3 counts yr^{-1} from the same deg would indicate a point source.)

Compact radio sources emit in bursts which almost certainly originate within regions smaller than a light day. Thus, a few years worth of neutrinos as computed from average luminosities could arrive within a few hours, making them much more easy to detect. It has been suggested¹⁶ that as many as 10^4 counts per 10^9 tons per burst could be recorded from a radio burst, for example, the nucleus of 3C120.

Table 1 Possible high energy neutrino sources

Site of He Particle production	Target material	Maximum expected counting rate from a typical source
Expanding supernova remnants	Ejecta, swept up matter, dense cloud from which star formed	10^2 per yr per 10^9 tons
Stellar explosions	Binary companion	$10^8/10^9$ tons per event
Pulsars	Binary companion, accreting matter young supernova remnant	10^3 per yr per 10^9 tons
Seyferts, radio galaxies, and QSOs	Clouds in nucleus, matter accreting on to compact object	10^3 per yr per 10^9 tons

Particularly significant is the fact that $\sim 10\%$ of the flux from Cen A is in the form of high energy γ rays. This corroborates the picture sketched above, that is that a significant fraction of the luminosity of a non-thermal galactic nucleus is caused by very energetic particles. The energy density in the Universe due to emission by Seyferts and QSOs has been conservatively estimated at $10^{-3} \text{ eV cm}^{-3}$. If Seyferts do emit $\sim 10\%$ of their luminosity in the form of high energy neutrinos, the energy density in such neutrinos could be as high as $10^{-4} \text{ eV cm}^{-3}$. Thus, one could reasonably hope to discover a cosmic background of high energy neutrinos standing out above the atmosphere at $E_\nu > 10^{12} \text{ eV}$.

Limits on deuterium production

Another area where neutrino astronomy may be critical is in constraining the astrophysical locations for producing deuterium¹⁷. If deuterium is only produced cosmologically then the Universe is open (see ref. 18 and refs therein). Epstein *et al.*¹⁹ showed that the only allowable alternative to the big bang for producing deuterium was extremely high energy particle interactions where most nuclei (in particular Li, Be and B) would be broken down to free nucleons. To avoid seeing the simultaneously produced γ 's from such interactions, any such production site must be hidden in an optically thick region (for example, Epstein²⁰ proposed the centres at quasars), however, neutrinos cannot be hidden in this way. Thus, limits on the neutrino flux would constrain, if not eliminate, any non big bang deuterium production site. The Reines experiment²¹ implies that the cosmic background at $E_\nu < 10^{12} \text{ eV}$ is well below the atmospheric background, and it can be shown²² that this constrains Epstein's model for deuterium production to $1 + Z > 2000(h^2\Omega)^{-2/3}$.

Conclusions

Many of the most exciting objects in the sky may be high energy neutrino emitters. Any object that emits γ 's by π^0 production will also naturally be a neutrino source. Thus, γ -ray astronomy gives us some degree of confidence that neutrino astronomy will not be a null field. However, the two fields are complimentary and will not merely duplicate each other because neutrinos are capable of being seen even when they are produced in regions which are optically thick to γ rays. For example, the very compact central regions of active galaxies, with their intense radiation fields, will be optically thick to γ -rays but can be

studied by neutrino astronomy. In probing these very central regions, one may discover how these objects are powered. In fact, even a null result is interesting because it would indicate the degree to which high energy particles are involved. In addition, γ -ray detectors mostly tend to be sensitive to lower energies, thus seeing an object in both ν 's and γ 's will tell us a lot about its high energy particle spectrum. (The mean free path of γ 's of energy $\geq 10^{14} \text{ erg}$ is $\approx 10^4 \text{ kpc}$).

One final advantage of ν -detectors is that they simultaneously observe a full 4π steradians. Thus they will not miss transient point sources. This feature may enable ν -detectors to alert other branches of astronomy to transient objects.

Whenever astronomers have opened a new channel for studying the Universe, new and unexpected sources have been discovered. One only has to consider, quasars and pulsars from radio astronomy, Cyg X1 from X-rays, and the still mysterious X-ray and γ -ray bursters to realise that the most exciting possible discoveries from ν -astronomy may not have been mentioned here.

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Rifting, crustal attenuation and subsidence in the Bay of Biscay

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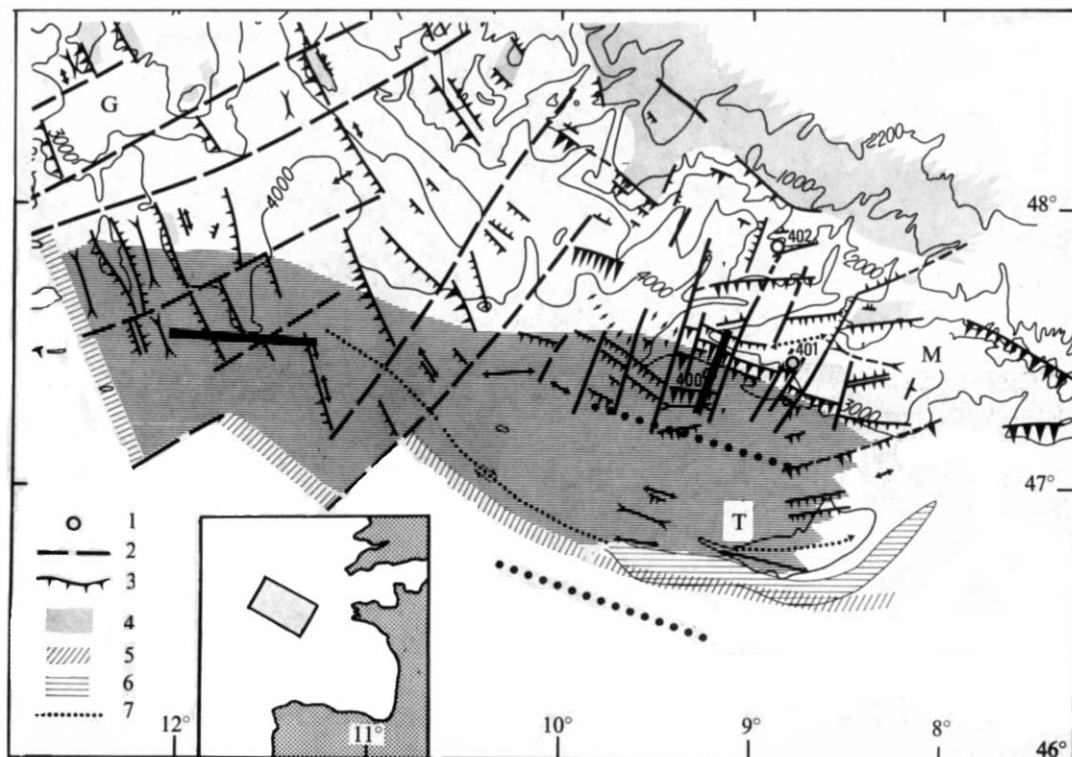
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The distribution of the Tertiary and Lower-Upper Cretaceous sediments in part of the northern continental margin of the Bay of Biscay, has been mapped. Three sedimentary sequences are indicated by the seismic profiles.

THE part of the northern continental margin of the Bay of Biscay discussed here intersects and lies seawards of the rifted Mesozoic-Cainozoic basin and Cornubian granite platform of the western approaches to the English Channel. The continental

slope is broad and marked by prominent features such as the Meriadzek Terrace, Trevelyan Escarpment and the Goban Spur. The structure of the margin consists of thin and unfaulted, Tertiary and Lower-Upper Cretaceous sediments that mantle a series of tilted and rotated fault blocks¹ underlain by a thinned continental crust^{2,3}. The distribution of these sediments and the fault blocks has been mapped using 10,000 km of multichannel seismic profiles occupied by the Institut Français du Pétrole (IFP-CNEXO-CEPM) and the Institute of Oceanographic Sciences for the Department of Energy (UK). These data were

Fig. 1 Schematic tectonic pattern of the rift system in NE Biscay: 1, sites 400, 401, 402; 2, faults; 3, lystric faults with arrows down dip; 4, Pre-Aptian erosional surfaces; 5, continental oceanic crusts boundary; 6, tectonised area along Trevelyan escarpment due to late Eocene compression; 7, late Eocene strike-slip fault or folds. Heavy dotted line, seismic refractions profile; heavy solid line, seismic reflection profile Figs 2a; 3. Horizontal hachures, areas where deep reflector 'S' can be seen.



acquired using 24-channel common depth point reflection techniques and processed using deconvolution before and after stack followed by the application of time and space variant filtering. Diffraction migration has also been applied to clarify reflections.

The seismic profiles show that the tilted and rotated blocks are bounded by lystric faults which delineate half-grabens created during rifting and subsequently covered by post-rift sediments^{1,4}. Three sedimentary sequences can be recognised on the seismic profiles. A pre-rift series which consists of thick layered sediments shown by parallel inclined reflectors is clearly visible within the fault blocks. Evidence for the age and depositional environment is given by drill and dredge data⁴⁻⁷. Site 401 of DSDP Leg 48 (refs 4, 5) penetrated Kimmeridgian–Portlandian limestones in the crest of one fault block although the regularity of the pre-rift reflectors (Fig. 2a) hints that substantial facies changes may be present. Dredging on exposed fault blocks at 4,200 m depth near Goban Spur, however, has yielded granites of Hercynian age⁷ and pebbles of Permo–Triassic (?) sandstones have been noted in Lower Cretaceous conglomerates⁶. The second syn-rift sequence, deposited contemporaneously in the half-grabens, is inferred to be pre-Aptian and probably post-late Jurassic⁴. The post-rift sequence penetrated at DSDP sites 400A, 401 and 402A was deposited after the end of rifting and the onset of spreading in Aptian time. It comprises thin pelagic or hemipelagic sediments of post-Aptian age that drape the underlying fault block topography⁴.

The tilted and rotated fault blocks can be followed from the shelf break to the outer part of the rise in the vicinity of the Trevelyan Escarpment at 4,500 m depth. The polarity of the faulting is consistently down towards the continent–ocean boundary and true horsts are rare. The fault blocks (Figs 1, 2a) typically trend WNW–ESE subparallel to the margin but their continuity is limited due to normal faults whose NE–SW and E–W trends may reflect the influence of older Hercynian structures such as the Armorican shear zones. Towards the Goban Spur, the fault blocks change trend to NNW–SSE and are paralleled by the magnetic anomalies of the adjacent ocean crust⁸. In both areas, the position of the continent–ocean boundary (Fig. 1) is clearly shown by a sharp change to a strongly diffracting opaque reflector that contrasts with the tilted reflectors observed within the fault blocks.

The fault blocks are typically spaced at between 10 and 30 km (Figs 1, 2a) and the bounding fault planes curve and flatten with depth. Throws of individual faults may be as much as 3–4 km. Comparable lystric faults have been described from the Viking Graben of the North Sea^{8,9} and from the Gulf of Suez rift¹⁰. Beneath the irregular surface defined by the crests of the fault blocks, a prominent reflector 'S' (Fig. 2b) is observed at depths between 9.0 and 11.0 s beneath the rise. Variations in the travel time to reflector 'S' beneath and between the fault blocks are produced by lateral variations in velocity. It may therefore be assumed that reflector 'S' is sub-horizontal and thus independent of the overlying faulted layer. Indeed, we have not observed any displacement of reflector 'S' even though the curved normal faults are associated with throws of as much as 3–4 km. An exactly similar feature observed at the western foot of Galicia Bank to the west of Portugal shows that reflector 'S' is not a local phenomenon (Fig. 4).

Further evidence on the nature of reflector 'S' is given by a refraction profile which crosses our seismic reflection profiles and was obtained by CNEXO using OBS (ref. 2). The base of the 4.9 km s⁻¹ layer is 9.5 km, close to the depth at which the lystric faults become horizontal. Below this layer, a 3-km thick layer with 6.3 km s⁻¹ occurs above the Moho (8.2 km s⁻¹). The refraction data indicate that the interface between the 4.9 and 6.3 km s⁻¹ layers lies at ~9.2 s, in reasonable agreement with the observed depth of reflector 'S' (Figs 2b, 3). The structure of the faulted layer is, however, complex, and horizontal inhomogeneities may be present. In view of this and the small 3-km thickness of the 6.3 km s⁻¹ layer the possibility exists that reflector 'S' may be the Moho. Clearly, further seismic studies are necessary.

Rifting and crustal attenuation

Experimental simulation of rifting using clay models has clearly shown that normally faulted tilted blocks are formed in response to crustal tension¹¹. The accommodation of these movements at depth bears directly on proposed mechanisms of crustal attenuation during rifting. One widely used model of rifting is based on analogues such as the East African rift system or Rhine graben.

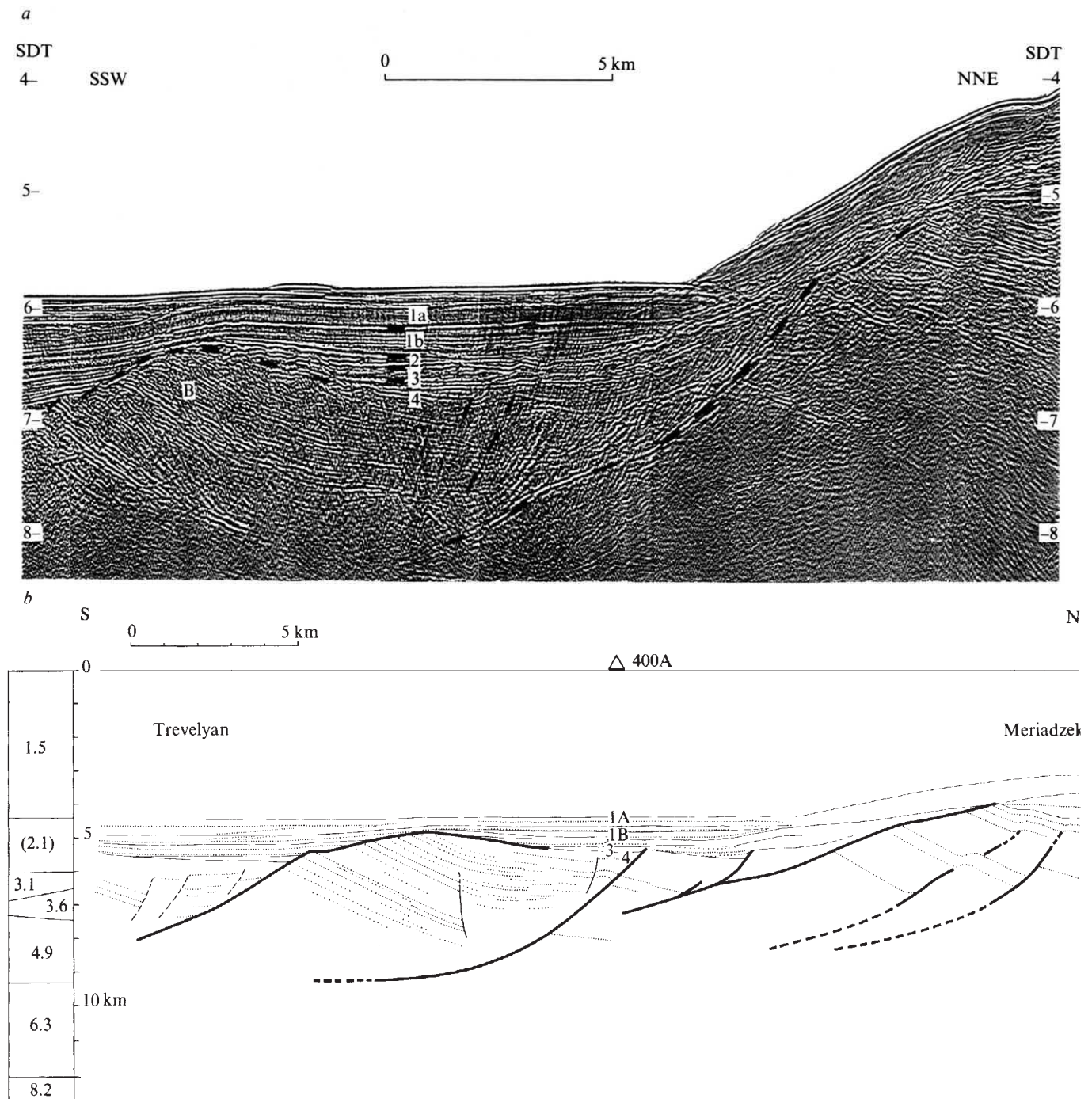


Fig. 2 *a* Tilted block and lystric fault. Acoustical basement B is constituted in a great part of layered sedimentary rocks of probable Jurassic and early Mesozoic age. These formerly continuous layers were faulted and tilted during the early Cretaceous rifting phase. Vertical scale in seconds, two-way travel time. Numbers 1a, 1b, 2, 3, 4 refer to overlying sedimentary formations: Profile OC 412, migrated; Site 400. *b*, Geological cross section through site 400 with same horizontal and vertical scale, constructed from seismic reflection—profile OC 412 migrated (see Fig. 1). Note the lystric faults bounding the tilted blocks. Near base of lystric faults is an horizontal reflector corresponding to interface between 4.9 km s⁻¹ and 6.3 km s⁻¹ layers defined by seismic refraction data. Moho discontinuity is at 12 km (ref. 24).

The following stages have been proposed¹²: upwarping or doming of the crust, incipient volcanic activity, formation of faults and increasing volcanic activity, subsidence of the graben(s). Other workers^{13,14} proposed that domal uplifts developed in isostatic response to mass deficiencies produced by partial melting of the mantle above rising plumes at the base of the lithosphere. In these models, sub-aerial uplift and erosion with 'sub-crustal erosion' are offered as a means of thinning the crust. The second and fundamentally different mechanism pro-

poses a combination of extension by brittle fracture in the upper part of the crust and ductile flow in the lower part of the crust¹⁵⁻¹⁹. Important constraints can be placed on these by the regional geology of the margin and also by the geometry of the extensional fault fabric.

In the Bay of Biscay, sub-aerial uplift which preceded rifting is not evident, in contrast to the classical models⁴. Rifting, which probably took place between late Jurassic and Intra-Aptian time⁴ occurred in a pre-existing epicontinental sedimentary

basin of Mesozoic age perhaps first formed during earlier Permo-Triassic distension^{5,9}. The seismic profiles indicate that no erosion occurred before and during rifting on the outer part of the margin, that is, in the axis of the rift system. Dredge data^{6,7}, the drilling results from Leg 48 (refs 4, 6) and the seismic data indicate that at the end of rifting, water depths were of the order of 2,000 m in the outer part of the margin. The existence of deep water marine beds implies that rifting was not associated with a substantial sub-aerial relief—a conclusion that can also be drawn for the Parentis Basin in south-east Biscay²⁰. We suggest that the rifting was not preceded by and is thus not a consequence of a domal uplift. Beneath the inner part of the margin, however, the crests of some tilted blocks are cut by a horizontal plane, indicating that some sub-aerial erosion did take place when the fault blocks were rotating.

The importance of normal faulting in thinning the crust has been more contentious, for little has been known of the geometry of such faults at depth and their relationship to the deeper structure of passive margins. The new data (Figs 2b, 5) clearly show that the lystric faults do not continue as far as the base of the crust but curve and flatten with depth demonstrating that extensional faulting is confined to the upper part of the crust. Such faults are considered to form by simultaneous faulting and tilting so that the fault blocks rotate towards the central subsiding trough and individual blocks dip away from the rift

if high pore fluid pressures are absent²², it seems unlikely that these would develop under an extensional stress regime.

Our hypothesis considers that the termination of faults with depth marks a fundamental transition in the rheological properties of the crust from brittle above to ductile below^{19,23,24}. The boundary or transition between them may be marked by the deep reflector 'S' so that the interval between the lystric faults and the Moho corresponds to a zone of ductile flow characteristic of the lower part of the crust. The hypothesis implies that there is a depth in a homogeneous crust subject to extension below which ductile behaviour occurs and above which elastic strain is released by periodic faulting. Flow within the ductile layer during rifting, that is, while the continental crust remains joined, is considered to accommodate the balance of the thinning required by the geological and geophysical data.

Our observations of rift tectonics in the Bay of Biscay support modifications to the Vening Meinesz hypothesis of rift valley formation made by various authors^{15-17,21,23-25}. Intraplate stresses arising from continental collisions or from small differences between the absolute velocities of plates may initiate stretching of the lithosphere^{10,14}. These stresses are imposed on a crust consisting of the brittle upper layer and a lower ductile layer that can deform rapidly when stress differences are large. As shown above, the limited extension and thinning produced by brittle fracture compared to the more important ductile thinning of the

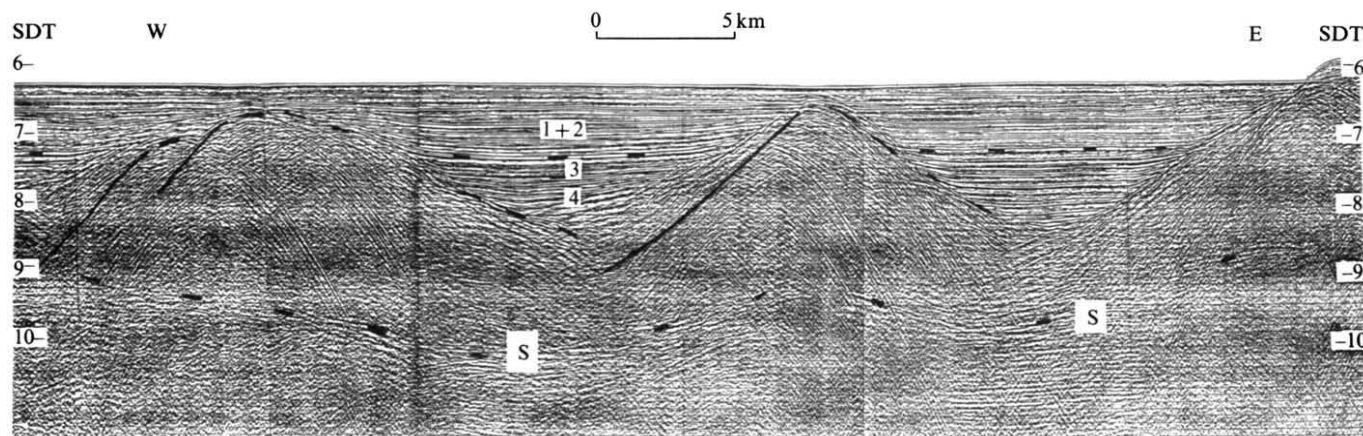


Fig. 3 Seismic profile south of Goban Spur showing tilted blocks with lystric faults. Note the horizontal reflector S below the tilted blocks. It is observed on the deepest part of the margin. On Trevelyan it corresponds to the boundary between a 4.9 km s^{-1} layer and a 6.3 km s^{-1} layer only 3 km thick. The Moho is around 12.5 km below sea level. Profile CM 16, processed.

axis²¹. Beneath the outer part of the margin, the continental crust affected by lystric faults is ~6 km thick although rotation of the blocks reduces this thickness to about 4–5 km (Figs 2b, 5).

As further fault movement becomes difficult when the fault plane becomes horizontal, we have roughly compared the crustal thinning and extension derived from the variable fault geometry with that given by gravity models and refraction data^{2,3}. For the typical rotation of 20–30°, extension of the area between the present shelf edge and the continent-ocean boundary is ~10% of the previous width. The rotation data also indicate a thinning of between 6 and 9 km (after ref. 21). The refraction data show, however, that the crust has thinned to 6 km (that is by 24 km assuming an original thickness of 30 km) beneath the outer part of the margin^{4,5}. We therefore conclude that extensional faulting in the upper part of the crust during rifting is inadequate to account for the observed thinning.

Clearly, the lower part of the crust must also be thinned but by some other mechanism. We have considered an origin by decollement, although this is not preferred, as both gravity and high frictional drag must be overcome to allow movement along a subhorizontal zone dipping toward the continent. Furthermore, the direction of movement would be opposite to that taking place during extension. Although frictional drag can be reduced

lower crust suggests decoupling between the brittle and ductile parts of the crust. Experiments in rock mechanics³⁰ have shown that the strain of ductile material increases continuously once a certain level of stress is reached (viscous behaviour). In contrast, continuous strain in the brittle material is limited because stress increases only until released by fracturing. These differences in rheological behaviour may imply that thinning of the ductile part of the crust began before the creation of the first fault in the brittle crust. As tensile stresses are the same at the lateral boundaries of the lithosphere, it follows that extension by continuous viscous flow initiated by a lower level of stress in the lower ductile part is more important than in the upper, brittle part. Normal faults develop as the strength of the brittle layer is exceeded and downfaulting results in a decrease in tensile stress and elastic shortening that equals the extension caused by faulting. Continued viscous flow in the ductile layer results in the accumulation of stress in the brittle layer that is relaxed by further faulting. The continuous necking of the lower ductile part of the crust is accompanied by a rise of the mantle and a development of a depression, the rift, in the brittle upper crust that allows continued rotation of the fault blocks along the lystric faults. It is important to realise that the mechanism allows greater thinning within the ductile layer than in the brittle layer

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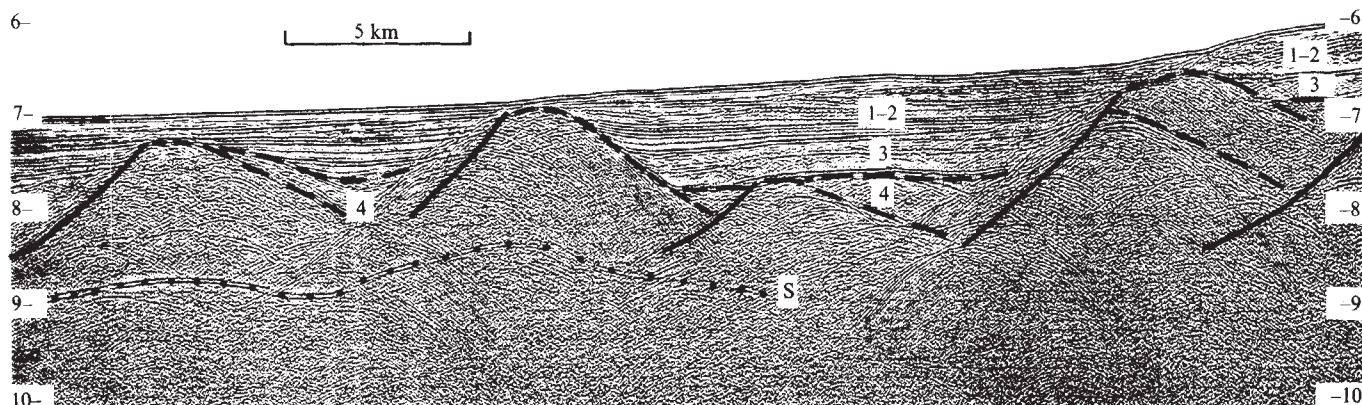


Fig. 4 Seismic reflection profile immediately west of Galicia Bank showing tilted blocks and lystric faults and an horizontal reflector below, as on Fig. 1(7); seismic formations 1-4 are the same as defined in Northern Biscay. Profile IFP-CNEXO-CEPM GP12 processed.

because of the difference in rheological properties. Extension within the brittle layer should not be regarded as a measure of thinning which largely takes place within the ductile layer.

Models suggested for the Baikal rift and Rhine Graben^{15,16} fit our observations well. Heat flow data for the Biscay margin²⁷ can only be interpreted in terms of thinning of the ductile lower part of the continental crust and not in terms of phase changes or intrusions. Rim uplifts predicted by the model may be due to regional isostatic adjustment indicated by the erosion of the fault blocks observed near sites 401 and 402 and more widely on the shelf. In Biscay, Galicia and the Aquitaine Basin^{1,4,20,26}, the regional geological data indicate that the rifting took place in a pre-existing epicontinental basin subject to subsidence and perhaps weakened during earlier rifting episodes. In these cases, previous crustal thinning which may have occurred in response to sediment loading as well as rifting may have subdued the development of complementary uplifts or erosion may have kept pace with uplift. The faulting may have been rapid although we cannot be specific on this point. For example, onlap of reflectors within the bedded sequence contained within the fault blocks is rare but thickening towards the axes of the half graben is common. This observation is indicative of relatively rapid and continuous contemporaneous movement rather than episodic movement.

Post-rifting subsidence

Regional subsidence of passive margins beginning at the onset of spreading and after rifting is a widely recognised phenomenon. In Biscay, post-rift subsidence of 2,300 m indicated by shallow water Aptian-Albian sediments was comparable to the subsidence produced by rifting^{4,5}. This subsidence was not caused by faulting, for the post-rift sediments are only rarely affected by faults. These exceptions are faults of late Eocene age developed along the Trevelyan Escarpment in response to the Pyrenean compression. The post-rift subsidence therefore took place by oceanwards tilting of the margin without decoupling of the continental and oceanic crust. Subsidence due to loading can be

neglected in Biscay as the post-rift sediments are thin (Figs 2b, 5).

The most widely accepted mechanism of post-rift subsidence is thermal contraction of the lithosphere initiated as the heat source migrates away from the margin at the onset of spreading; the exponential decrease in subsidence with time is considered to be similar to that deduced for the ocean crust^{4,28}. The depth of deposition of Aptian sediments (Fig. 6) that is, of the topography of the margin at the end of rifting, has been reconstructed from the new seismic and palaeobathymetric data⁴. Although the present altitude of a point on the margin depends on its initial altitude, the curve shows that the subsidence increases progressively from the shelf towards the deep ocean. Variations in

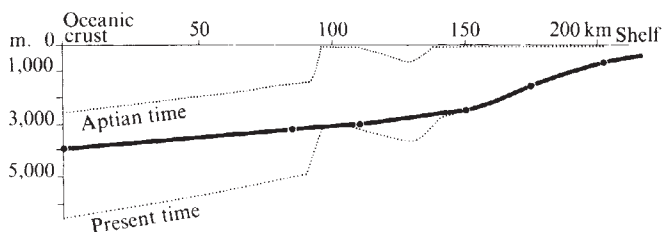
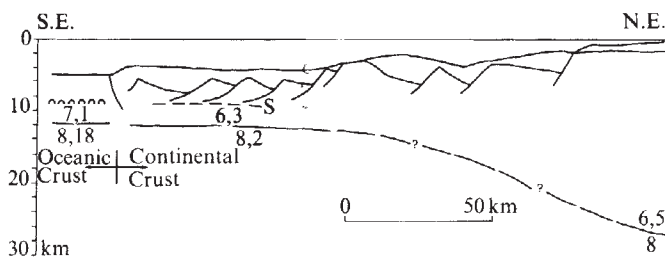


Fig. 6 Absolute amount of post-rifting subsidence of N. Biscay margin from the shelf to the oceanic crust. Upper dotted line, simplified topography of the rift at Aptian time; lower dotted line, present depth of Aptian corrected for loading; solid line, amount of subsidence.

subsidence rate cannot be defined with precision, however, because palaeontologically determined depths become increasingly inaccurate for increasing water depths and age. Nevertheless, data⁴ do not contradict an exponential decrease in subsidence. The subsidence of the thinned continental crust adjacent to the continent-ocean boundary is greatest and most similar to the ocean crust, whereas that of the thick crust beneath the shelf is least and most different. The magnitude of the subsidence is thus clearly related to crustal thickness. Change of slope on the subsidence against distance curve at 150 km (Fig. 6) may reflect abrupt changes in crustal thickness. The curve also substantiates the coupling of the continental and oceanic crust because subsidence of the oceanic crust is approximately equal to the continental oceanic crust.

Unfortunately, we cannot address the role of the ductile layer, Moho and Upper Mantle on the subsidence during and after rifting. Their nature and position may well have been different during and after rifting. Nonetheless, spatial variations in the post-rift subsidence of the passive margins of Biscay are at least a partial function of crustal attenuation produced by thinning of the ductile layer during rifting.

Fig. 5 Schematic crustal sections through the N. Biscay margin (data refs 2, 19).



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A new Thy-1 alloantigen as a temporal marker of T lymphocyte differentiation

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A new Thy-1 alloantigenic specificity is defined in the mouse. Antigen expression is restricted to 'mature' and memory T cells, with either helper or killer activity.

THE importance of lymphocyte differentiation antigens in defining the cellular heterogeneity of the immune system is now well established; functionally distinct lymphocyte populations exhibit a unique surface antigen phenotype, and may be enriched or depleted with specific antibody¹. In the mouse, the initial classification of lymphocytes as B or T cells was based on surface expression of immunoglobulin or the Thy-1 alloantigen^{2,3}. Subpopulations of T cells have since been defined. These are distinguished by their differential expression of the alloantigens Ly-1, Ly-2 and Ly-3 (refs 4–6). Studies by Cantor and Boyse with the Ly markers indicate two independent pathways for the differentiation of T lymphocytes^{7,8}: thymocytes and 'precursor' T cells with a Ly-1⁺ 2⁺ 3⁺ surface phenotype generate either helper cells (Ly-1⁺ 2⁺ 3⁻) or suppressor/killer cells (Ly-1⁻ 2⁺ 3⁺), commitment to either differentiation pathway preceding exposure to antigen. However, virgin and memory T lymphocytes cannot be distinguished by means of these markers.

Evidence is presented here for a new Thy-1 alloantigen which may be used to distinguish virgin and memory T cells. The antigen is absent from neonatal T cells but present on 50% of the adult T cell population. Functional tests indicate that this new specificity is restricted to 'mature' and memory T cells, with either helper or killer activity.

Xenoantisera to Thy-1

Experiments with xenoantisera to murine tissues provided the first, fortuitous indication that Thy-1 included an hitherto unrecognised specificity. Rats and AKR mice both express the Thy-1.1 alloantigen on brain and thymus, and reciprocal immunisation should only elicit antibody to xenogeneic determinants. It was unexpected to find that rat antisera to AKR brain defined a subpopulation of T lymphocytes (Fig. 1).

Xenoantiserum dilutions of log₃ 1–3 and C' (guinea pig complement) lysed a constant number of cells in AKR thymus (75–80%), lymph node (30–35%) and spleen (18–20%),

without affecting bone marrow cells (<4%). (Values are also included in Fig. 1 for lysis by Thy-1.1 alloantibody and C'). Cytotoxicity was removed by absorption with AKR brain, but not rat brain or thymus, thereby excluding autoantibody activity.

Identical values were obtained with lymphoid tissues of Thy-1.2-positive mice: CBA, C57BL/6, BALB/c, LPJ, C3H/he and A strains. However, spleen cells from outbred, athymic nu/nu mice were not lysed (<3%), suggesting specificity for a subpopulation of T lymphocytes (about 50% of Thy-1⁺ cells in spleen and lymph node, Fig. 1). This was confirmed in experiments with mitogen-treated cultures of CBA spleen. Xenoantiserum (1/3 to 1/9 dilution) and C' lysed concanavalin A-induced T lymphoblasts (35–50%) and phytohaemagglutinin A-induced T lymphoblasts (18–25%) but not lipopolysaccharide-induced B lymphoblasts (<4%) at the peak of the mitogenic response (48–72 h).

The number of peripheral lymphocytes lysed by xenoantiserum and C' showed a striking change with age. There was no appreciable lysis by xenoantiserum during the first 2 weeks of

Table 1 Indirect immunofluorescent staining of CBA spleen cells with xenoantibody or alloantibody after capping of surface immunoglobulin

	Fluorescent cells (% ring staining)
Anti-BAT	25
Anti-Thy-1.2	41
Anti-Thy-1.2 ab	22
FITC-anti-mouse IgC	4
FITC-anti-rat IgC	3

CBA spleen lymphocytes ($5-10 \times 10^7$; 3 ml) were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (Miles; 1/20 dilution) for 2 h at 37°C (minimum time for complete capping) and washed twice with buffered saline. Aliquots ($2-4 \times 10^6$; 100 μ l) were then incubated with an equal volume of anti-BAT (1/3–1/12 dilution) or anti-Thy-1.2 ab (1/10–1/30 dilution) or anti-Thy-1.2 (1/10–1/30 dilution) for 30 min at 3°C, washed and incubated for 30 min at 3°C with 50 μ l of FITC-conjugated rabbit anti-rat IgG or FITC-conjugated rabbit anti-mouse IgG. Cells were washed and examined with a Leitz Ortholux fluorescence microscope.

life (Fig. 2; <4%). Moreover, absorption of antiserum with newborn CBA spleen (1: 1, v/v; 2 h at 3 °C) did not alter the cytotoxic titre for adult spleen or lymph node, thus indicating absence of a particular cell population from the neonate. However, the adult number of T cells were lysed by Thy-1 alloantibody within a day of birth. Henceforth, the antigen(s) recognised by rat anti-AKR brain sera will be referred to as 'BAT' (brain-associated thymus antigen).

Effect of thymectomy

The neonatal mouse is immunologically incompetent and surgical removal of the thymus drastically, and permanently, impairs homograft immunity, whereas thymectomy at 3 weeks has little effect^{9,10}. However, the proportion of Thy-1⁺ cells in peripheral

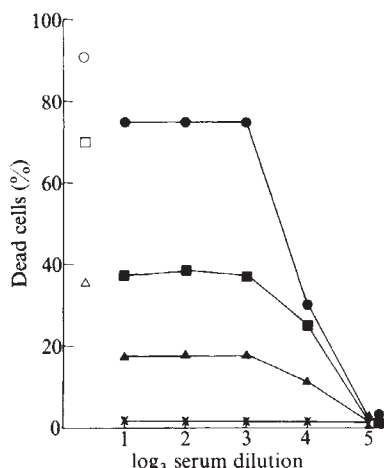


Fig. 1 Cytotoxic titre of rat anti-AKR brain serum and C' for AKR lymphoid cells: thymus (●), lymph node (■), spleen (▲) and bone marrow (×). Values are also included for lysis of thymus (○), lymph node (□) and spleen (△) by anti-Thy-1.1 (1/16 dilution: Searle Diagnostic) and C'. Rats, either outbred Wistar or inbred PVG and August strains, received five weekly intraperitoneal injections of AKR brain homogenate (1:1, v/v; phosphate-buffered saline, PBS). Sera from 6–8 rats were pooled, heat-inactivated and absorbed with mouse liver homogenate to remove species antibody (1:1, v/v; 2 h at 3 °C). Viable lymphocytes (>98%) were obtained by Ficoll-Hypaque centrifugation of tissue cell suspensions. In the cytotoxic assay, equal volumes of cells (2.5×10^6 ; 50 μ l), xenoantiserum and C' (absorbed with Agarose) were incubated at 37 °C for 30 min and washed with PBS (2 ml). A two-step incubation was used for anti-Thy-1.1 to avoid the anti-C' activity of mouse serum. Cell viability was estimated by dye exclusion using cosin (0.1%, w/v). Control values for normal rat serum (absorbed with mouse liver) and C' were <4% dead cells.

lymphoid tissues is identical in the neonate and adult (ref. 11, Fig. 4a), perhaps indicating further, thymus-dependent, maturation of the peripheral T cell in the first 2 weeks of life.

The percentage of cells reacting with xenoantibody showed an abrupt increase during this time (Fig. 2). An attempt was made to determine whether thymectomy affected the appearance of the BAT⁺ cell population. CBA mice were thymectomised at 3 d or at 3 weeks, and killed at weekly intervals thereafter; pooled spleen cells from six or more donors were treated with xenoantibody or anti-Thy-1.2 and C'. Neonatal thymectomy delayed, but did not prevent the appearance of BAT⁺ cells (Fig. 3a). However, adult thymectomy produced a reciprocal decrease in the Thy-1⁺ cells and increase in BAT⁺ cells, so that at 5 weeks post-thymectomy these populations were numerically equivalent (Fig. 3b).

Dual specificity of Thy-1 alloantisera

The existence of a new murine antigen (BAT) unrelated to Thy-1, but restricted to brain and the T cell series seems unlikely. Alternatively, Thy-1 itself might define two serological determinants differing in temporal expression: 'conventional'

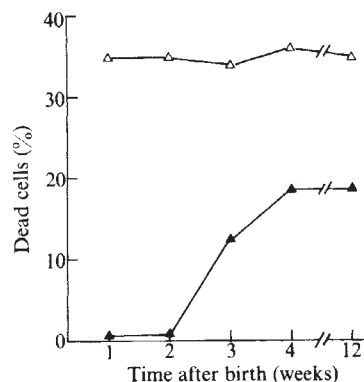


Fig. 2 Cytolytic effect of rat anti-AKR brain serum (▲, 1/6 dilution) or anti-Thy-1.2 (△, 1/16 dilution) and C' on CBA spleen cells of different ages.

Thy-1 present on neonatal and adult T cells, and a second specificity restricted to the adult. Alloantisera might then be expected to contain dual specificities, distinguished only by their reactivity with neonatal T cells.

To test this possibility, alloantisera (C3H anti-AKR, or AKR anti-C3H) were absorbed with 2–3-d-old spleens from either AKR or CBA mice and examined for residual activity. Absorption of Thy-1.2 (Fig. 4b) or Thy-1.1 (Fig. 4c) alloantisera had no effect on the cytotoxic titre for adult tissues but removed all activity for neonatal spleen. The age of the spleen cells used for absorption was critical; 8–10-d-old donor cells removed all cytolytic activity.

Plateau values for the percentage of cells lysed (thymus, spleen and lymph node) were obtained with alloantiserum dilutions of $\log_2$ 2–6 (Fig. 4a; cytotoxic index and titre of anti-Thy-1.1 identical, data not presented). Absorption reduced these values for each tissue to give profiles strikingly similar to those obtained with xenoantisera (Fig. 1); thymus 80%, spleen 18–20%, lymph node 30–35%. Adult AKR thymus (Fig. 4c; 50%) was the obvious exception. These results suggested that xenoantisera and absorbed alloantisera were recognising the same cell population. To confirm this, cytotoxic values were obtained after consecutive addition of alloantisera and C', and xenoantiserum and C', to CBA or AKR spleen, or lymph node cells. The number of cells lysed remained unaltered after addition of xenoantiserum.

Murine alloantisera are frequently contaminated with viral antibody, leading to some confusion over serological specificity. It might be argued that the lymphocytotoxic antibody, removed by absorption with neonatal spleen, was directed against viral determinants, residual activity being *bona fide* Thy-1 antibody (Fig. 4b, c). This argument presupposes the absence of T cells in neonatal spleen; this is unlikely, as monoclonal Thy-1.2 antibody and C' lysed 30–35% CBA spleen cells from both 3-d-old and adult donors. Also, xenoantiserum and absorbed Thy-1.1 alloantibody lysed an equivalent number of spleen cells (20%) in

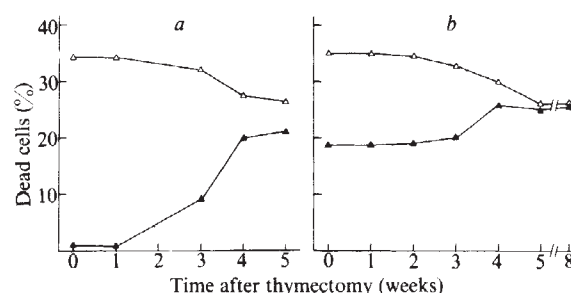


Fig. 3 Effect of neonatal (a) or adult (b) thymectomy on the number of CBA spleen cells lysed by rat anti-AKR brain serum (▲, 1/6 dilution) or alloanti-Thy-1.2 serum (△, 1/16 dilution) and C'.

congenetic A strain mice, where Thy-1.1 occurred on a different genetic background.

Lymphocytotoxicity compared with immunofluorescence

Lymphocytotoxic tests often give spurious results due to the presence of non-complement-fixing antibodies. Failure to lyse all T cells with xenoantibody or absorbed alloantibody might be an artefact of C' fixation, and misinterpreted as a new serological specificity. It was necessary, therefore, to confirm these estimates by a different technique. Indirect immunofluorescence is a reliable method of detecting cell-bound antibody, although staining of lymphocytes with alloantibody or rat antibody includes a high background staining of immunoglobulin-bearing B cells. This background may be reduced by an initial 'capping' of B cells with antiglobulin.

Reasonable agreement was obtained by indirect immunofluorescence and no more than 20% of spleen cells bound xenoantibody or absorbed alloantibody. Both tests, therefore indicate that the sera are specific for a subpopulation of T cells.

Significance of a second Thy-1 alloantigen

We have shown that Thy-1 alloantisera contain two distinct antibody specificities, distinguished by absorption with neonatal T cells, whereas xenoantisera recognise a Thy-1 determinant restricted to the adult T cell.

The *Thy-1* locus, *Thy-1^a* and *Thy-1^b* alleles, is on chromosome 9 of the mouse, and there is no evidence of polymorphism¹². Biochemical studies in the rat, however, indicate molecular heterogeneity of Thy-1. Williams *et al.*, using lectin-affinity chromatography, have resolved Thy-1 from rat lymphocytes into two components with molecular weights of 25,000 and 27,000 (ref. 13). Differences in lectin affinity were attributed to the carbohydrate moiety rather than the polypeptide. It is reasonable to assume that the dual specificities, defined here, also reflect post-translational modification of Thy-1, possibly by glycosylation. It may be anticipated that monoclonal Thy-1 antibodies, produced by hybridoma cell lines, will be specific for either 'conventional Thy-1' or the 'alternative Thy-1' defined here.

We describe below our attempts to attribute a function to the T cell population reacting with xenoantibody (anti-BAT), and the alloantibody, absorbed with neonatal spleen cells (anti-Thy-1 ab).

Phenotype of helper T cells

Helper T cell function was assayed by adoptive cell transfer. Spleen cells from normal or adult-thymectomised mice were transferred, together with sheep red blood cells (SRBC) as

immunogen, into lethally irradiated syngeneic mice; recipients were assayed for anti-SRBC plaque-forming cells (PFC) after 6 d.

The anti-SRBC response requires helper T cell cooperation. Pretreatment of donor lymphocytes with anti-Thy-1 and C' abolished the development of direct and indirect PFC (Table 2, group 1). However, removal of >50% of T cells with anti-BAT or anti-Thy-1 ab and C' had no effect on the primary anti-SRBC response, and even elevated the number of indirect PFC. Spleen cells transferred from mice, adult-thymectomised 4 months previously, developed a reduced number of PFC in irradiated hosts (Table 2, group 2). Here, in contrast, the number of PFC was reduced even further by anti-BAT or anti-Thy-1 ab and C'. Cells from normal mice, primed 4 months earlier with SRBC, developed an increased number of PFC (Table 2, group 3). Treatment with anti-BAT and C' now reduced the number of indirect PFC but had no effect on direct PFC. These results indicate that helper cells in the primary response to SRBC are BAT⁻ Thy-1 ab⁻, but BAT⁺ Thy-1 ab⁺ in thymectomised mice; in a secondary response, only a proportion of helper cells are BAT⁺.

The increased number of indirect PFC from the virgin donors treated with antibody and C' may be due to elimination of suppressor T cells, but as we have no experimental system to generate or estimate such, this cannot be confirmed.

Phenotype of killer T cells and their precursors

Two experimental protocols were used: either an adoptive cell transfer of CBA lymphocytes into lethally irradiated F₁ (CBA × DBA/2) recipients (*H-2^{k/d}*) to generate alloreactive (*H-2^d*) killer cells, or challenge of normal or adult-thymectomised, CBA mice with allogeneic tumour. Killer activity was assayed by the chromium release method using the P815Y tumour as a target.

Pretreatment of donor lymphocytes, or recipient F₁ cells at the time of assay, with anti-BAT or anti-Thy-1 ab and C' had no effect on killer activity, indicating that neither precursor nor effector cells expressed the antigen(s) (Table 3, group 1). However, contradictory results were obtained in the second series of experiments (Table 3, group 2). Normal CBA mice were immunised with allogeneic tumour and killer activity was assayed at various times thereafter. Optimum killer activity was generated at 12 d; between 12 and 18 d ⁵¹Cr release was unaffected by antibody and C', whereas at 22 or 35 d values were reduced to base line.

This finding was constantly reproducible. Killer cells became susceptible to cytotoxic treatment with antibody and C' at 20–23 d following immunisation. In CBA mice thymectomised as adults and challenged with allogeneic tumour 5 weeks later,

Fig. 4 Cytotoxic titre of Thy-1 alloantisera before (a, anti-Thy-1.2) and after (b, anti-Thy-1.2; c, anti-Thy-1.1) absorption with neonatal spleen. Alloantisera (Searle Diagnostic) were absorbed (1/4 dilution) with an equal volume of packed neonatal spleen cells, either CBA or AKR (1:1, v/v; 2 h at 3 °C) and assayed for residual cytotoxic activity: adult thymus (○), lymph node (□), spleen (▲), neonatal spleen (△).

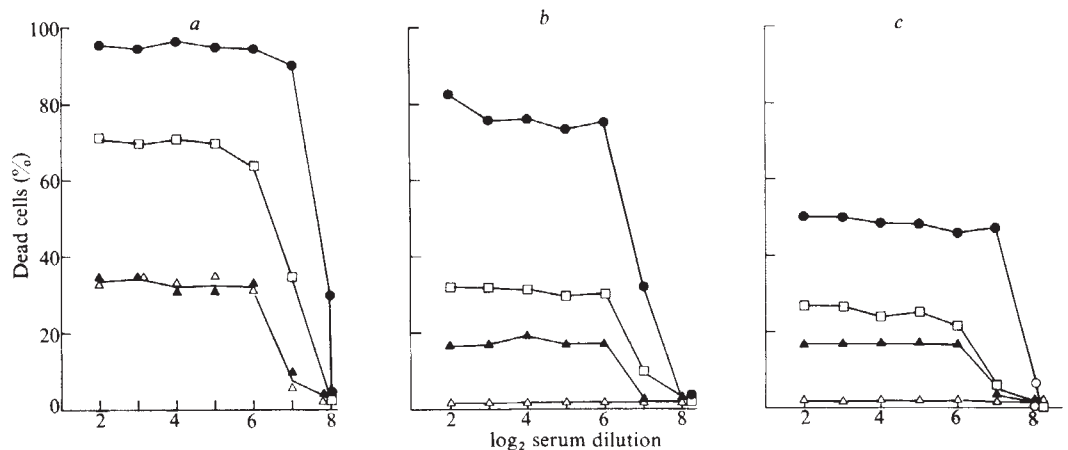


Table 2 Effect of xenoantibody or alloantibody and C' on the generation of a primary and secondary antibody response to SRBC in irradiated hosts

Group	Cell source	Direct PFC per 10 ⁶ cells	Indirect PFC per 10 ⁶ cells
1	Spleens of virgin CBA mice, treated with*:		
	NRS+C'(<2%)*†	1,193 (898–1,439)	1,781 (1,635–2,126)
	Anti-BAT+C'(20%)*†	1,524 (1,111–2,083)	3,016 (2,142–3,640)
	Anti-Thy-1.2+C'(32%)*†	138 (90–221)	293 (192–442)
	Anti-Thy-1.2 ab+C'(18%)*†	1,030 (972–1,094)	1,736 (1,370–2,187)
2	Spleens of adult-thymectomised CBA mice, treated with*:		
	NRS+C'(<2%)*†	296 (257–358)	920 (708–1160)
	Anti-BAT+C'(24%)*†	127 (107–145)	205 (187–237)
	Anti-Thy-1.2 ab+C'(21%)*†	81 (64–116)	237 (209–262)
3	Spleens of primed CBA mice, treated with*:		
	NRS+C'(<1%)*†	3,299 (2,931–3,742)	28,666 (25,000–33,250)
	Anti-BAT+C'(18%)*†	3,517 (3,302–3,716)	14,020 (13,096–14,721)
	Anti-Thy-1.2+C'(34%)*†	673 (531–789)	5,035 (3,986–5,580)

Spleen cells from six or more mice were pooled and assayed for PFC by the slide method, as described by Dresser¹⁹. Values for PFC per 10⁶ spleen cells represent the mean and range for six individual determinations.

* Viable lymphocytes were obtained by Ficoll–Hypaque centrifugation of CBA spleen cells. An equal volume of cells (4×10^8 ; 1 ml), C' and normal rat serum (NRS, 1/3 dilution), or anti-BAT (1/3 dilution), or anti-Thy-1.2 (1/8 dilution), or anti-Thy-1.2 ab (1/8 dilution) were incubated for 30 min at 37 °C. Lymphocytes (2×10^7 ; 0.3 ml) and SRBC (10^8) were injected intravenously into irradiated (900 R) CBA mice. Recipient mice were killed after 6 d.

† Number of cells lysed by antiserum and C' before adoptive transfer.

killer activity was always eliminated by anti-C' antibody (Table 3, group 3).

CBA mice are exceptional; their killer cells express a Ly-1⁺2⁺3⁺ phenotype whereas most other strains, including C57BL/6, are Ly-1⁺2⁺3⁺ (ref. 14). Therefore, the experiments were repeated with C57BL/6 mice (Table 3, group 4) with similar results: killer activity was eliminated by anti-BAT and C' at 22 d but not at 12 d. One may conclude that pre-killer and killer T cells are BAT⁺, Thy-1 ab⁺, whereas memory killer cells are BAT⁺ Thy-1 ab⁺.

A two-compartment model of antigen expression

Figure 5 schematically summarises our results. Two compartments for the peripheral T cell population are defined antigenically, BAT⁺ Thy-1 ab⁺ and BAT⁺ Thy-1 ab⁺. These antigens are distinct from any other lymphocyte differentiation antigen so far described in that they are markers of time not function. This may allow the estimation of transit time between

two cell compartments: virgin (or primary effector) cells and mature (or memory) cells. Lymphocytes leaving the thymus are BAT⁺ Thy-1 ab⁺. Removal of the source of precursor cells (by adult thymectomy) produces a majority of BAT⁺ Thy-1 ab⁺ peripheral T cells within 30–35 d (Fig. 3, Tables 2, 3).

The time required to generate memory killer T cells may also be estimated. After immunising normal CBA mice with allogeneic tumour, T killer cells are initially BAT⁺ Thy-1 ab⁺ (Table 3; 12–18 d) but within 20–23 d killer activity is present in the BAT⁺ Thy-1 ab⁺ population. In adult-thymectomised mice, killer cells are BAT⁺ Thy-1 ab⁺ at all times.

Raff and Cantor have proposed two major categories of peripheral T cells¹⁵. The T₁–T₂ concept was a useful working model which gave some rationale to earlier reports of synergy between different Thy-1⁺ cell populations^{16,17}. T₁ cells were defined as short-lived, non-recirculating lymphocytes that were susceptible to adult thymectomy. T₂ cells were long-lived, recirculating lymphocytes; they were resistant to adult thymectomy but highly sensitive to the *in vivo* effect of anti-lymphocyte serum. This T₁–T₂ classification is somewhat overshadowed by

Table 3 Effect of xenoantibody or alloantibody and C' on the generation and activity of alloreactive, killer T cells

Group	Cell source	Assay time (d)	⁵¹ Cr release (%)§ NRS + C'	Anti-BAT + C'	Anti-Thy-1.2 + C'	Anti-Thy-1.2ab + C'
1.	Adoptive transfer (CBA → CBA/DBA-2)					
	Pre-killer cells*	7	57	54	11	ND
	Killer cells†	7	53	60	8	57
2.	Allogeneic response: normal CBA mice‡	12	49	53	12	49
		14	68	64	12	66
		18	75	66	10	63
		22	70	11	8	10
		35	62	12	11	9
3.	Allogeneic response: adult thymectomised mice‡	12	52	13	9	13
		22	60	10	10	12
4.	Allogeneic response: normal C57BL/6 mice‡	12	78	71	13	ND
		22	68	10	10	ND

* Viable lymphocytes, obtained by Ficoll–Hypaque centrifugation, were treated with antiserum and C', as in Table 1. Donor lymphocytes (2×10^7 ; 0.3 ml) were injected intraperitoneally into irradiated (900 R) F₁ hosts.

† Irradiated F₁ recipients were killed at 7 d; spleen cells were treated with NRS, or antiserum, and C' and assayed for killer activity.

‡ Mice were injected intraperitoneally with 10^7 P815Y tumour cells and killed at various time intervals (12–35 d). Ficoll–Hypaque-purified spleen cells were treated with NRS, or antiserum, and C' before assaying for killer activity.

§ Spleen cells (2×10^7 /ml) were incubated with ⁵¹Cr-labelled tumour (2×10^5 /ml) for 5 h at 37 °C, centrifuged, and radioactivity in the supernatant determined. Values are given for % of total radioactivity in the supernatant; complete target lysis is equivalent to 75–80% release. Spontaneous release of ⁵¹Cr, in the absence of spleen cells, was 8–12%. ND, not determined.

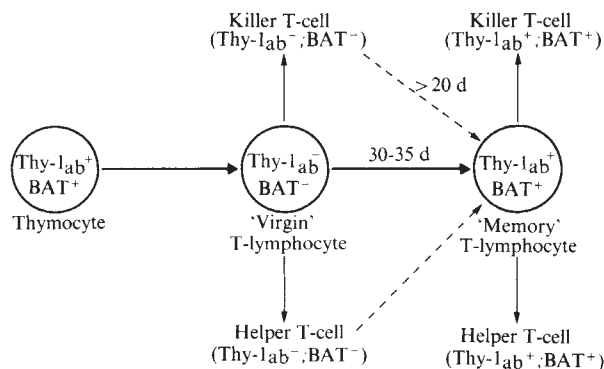


Fig. 5 A scheme of differential antigen expression by murine lymphoid cells.

recent studies with the Ly alloantigens^{7,8}; however, in functional terms, the T_2 population includes both $Ly-1^+$ helper and $Ly-2^+3^+$ suppressor/killer cells, and T_1 represents the precursor population of $Ly-1^+2^+3^+$ cells.

Some analogies may be made between $Ly-1^+2^+3^+$ (or T_1) lymphocytes and the BAT^- $Thy-1\ ab^-$ population (Fig. 5). In the neonate, T cells are exclusively $Ly-1^+2^+3^+$ and BAT^- $Thy-1\ ab^-$ (Figs 2, 4b, c) and both account for 50% of the adult population (Figs 1, 4b, c); the numbers of $Ly-1^+2^+3^+$ cells and BAT^- $Thy-1\ ab^-$ cells (Fig. 3b, Tables 2, 3) are decreased after adult thymectomy⁷. The $Ly-1^+2^+3^+$ phenotype, however, defines a precursor population that differentiates further to either helper ($Ly-1^+2^-3^-$) or killer ($Ly-1^-2^-3^+$) cells^{7,8}. The BAT^- $Thy-1\ ab^-$ population includes precursor cells and primary effector cells, and antigen expression reflects time not function.

The above model (Fig. 5) presents an obvious paradox. In the adult mouse, equal numbers of peripheral T cells are BAT^- $Thy-1\ ab^-$ and BAT^+ $Thy-1\ ab^+$; both populations can generate killer cells and helper cells (Tables 2, 3). However, the effector cells in a primary immune response are exclusively BAT^- $Thy-1\ ab^-$. Following adult thymectomy, effector cells are BAT^+ $Thy-1\ ab^+$ and helper function is somewhat reduced. These findings imply that, during a primary immune response, there is

a selective recruitment of 'young' T cells which have just left the thymus.

Certain reservations must be made concerning helper function. Further experiments are required with limiting numbers of T cells, for both the primary and secondary antibody response of irradiated hosts, to demonstrate conclusively that most helper cells are BAT^- $Thy-1\ ab^-$.

If this scheme is essentially correct and there is recruitment of young T cells, several predictions can be made and tested experimentally. For example, the ageing mouse shows a progressive decline in thymic function and a reduced antibody response¹⁸. The age-dependent changes in humoral immunity may mirror, or even be a consequence of, altered ratios in the two T cell compartments, in which case, old mice might resemble adult-thymectomised mice.

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A suppressor mutation in the nematode acting on specific alleles of many genes

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A suppressor mutation has been isolated in Caenorhabditis elegans through reversion analysis of a muscle-defective mutant. The suppressor mutation acts on specific alleles of at least six genes and in one case we have been able to show that it partially restores functional gene product to a mutant otherwise lacking that product. These and other features of the suppressor suggest that it acts at some step in information transfer, perhaps through mechanisms similar to those described previously in microorganisms.

IN intergenic suppression, the effects of a mutation in one gene are alleviated by mutations in other genes. Many different suppressor genes have been found in microorganisms, where restoration of the wild phenotype is achieved by a variety of mechanisms. Some suppressor genes compensate for the ori-

ginal mutation by affecting subunit interactions in macromolecular assemblies¹⁻⁴. Others act more indirectly by providing alternative biochemical pathways⁵⁻⁷ or by changing the physiological balance of the cell^{8,9}. A particularly important class is that of informational suppressors (for review see refs 10-13), which alter the fidelity of translation of mutant messages to allow the production of functional gene product, by changes in either transfer RNA¹⁴⁻¹⁹ or some other component of the translation machinery¹¹.

The identification and analysis of suppressor genes in microorganisms have provided an approach to problems that were not readily accessible with other methods of study. In multicellular organisms, the analysis of mutants and their suppressors might lead to the identification of the primary products of genes participating in developmental pathways and thereby provide some hint of the molecular interactions involved in this complex

Table 1 Summary of the results of four 3-factor crosses used to locate *sup-5** in LGIII

Cross	Parental genotype			Number of each recombinant genotype obtained	
	<i>e1464</i>	+	+	<i>e1464 + dpy-18</i>	<i>+ + dpy-18</i>
1	+	<i>unc-69</i>	<i>dpy-18</i>	10	0
2	<i>e1464</i>	+	<i>dpy-18</i>	<i>+ unc-69 dpy-18</i>	<i>+ + dpy-18</i>
	+	<i>unc-69</i>	+	12	8
3	<i>e1464</i>	+	+	<i>e1464 + dpy-18</i>	<i>+ + dpy-18</i>
	+	<i>unc-32</i>	<i>dpy-18</i>	21	0
4	+	+	<i>e1464</i>	<i>lon-1 + e1464</i>	<i>lon-1 + +</i>
	<i>lon-1</i>	<i>dpy-19</i>	+	6	0

To establish linkage, 20 suppressed animals were picked from among the progeny of hermaphrodites of the genotype *unc-15/+*; *m/+*; *sup-5*+, with the marker (*m*) being tested *trans* to the suppressor. The progeny of these suppressed animals were inspected to confirm that the parent was homozygous for the suppressor and for the presence of the marker. In all cases, except when markers from LGIII were used, the progeny of approximately 2/3rds of the parents included *m* animals. With markers from LGIII, many fewer parents contained the marker mutation. The mutant alleles used in the crosses were *unc-69*(*e587*), *dpy-18*(*e364*), *dpy-19*(*e1259*) and *lon-1*(*e185*)³⁶. For the 3-factor crosses, appropriate double mutants were constructed and mated with males carrying the third mutation to obtain the hermaphrodites listed under parental genotype. Among the progeny, animals with a recombinant phenotype were selected. In crosses 1, 2 and 3, dumpy animals were picked and progeny of these animals examined to determine the recombinant genotype, taking advantage of the observation that *dpy-18*(*e364*) *sup-5*(*e1464*)/*dpy-18*(*e364*) + animals are partially suppressed but still obviously dumpy and that *dpy-18*(*e364*) *sup-5*(*e1464*) homozygotes are nearly fully wild. From cross 4, long recombinants were selected, as *dpy-19* is epistatic to *lon-1*. Strains homozygous for the recombinant chromosome were obtained, and the presence of the suppressor was ascertained by testing with *unc-15*(*e1214*).

* Gene names in *C. elegans* consist of an italicised 3-letter abbreviation for a general phenotypic character, (for example, *unc* for uncoordinated, *dpy* for dumpy and *sup* for suppressor) separated by a dash from an italicised number to denote the specific gene, for example, *sup-5*. When appropriate an italicised roman numeral may follow, to indicate the linkage group of the gene, for example, *sup-5 III*. Mutation names consist of 1 or 2 lower case italicised letters (used to indicate the laboratory of origin) followed by an italicised number, for example, *e1464*. *e* denotes mutations from the Cambridge collection and *st* denotes mutations from the St Louis collection. When gene and mutation names are used together the gene name is followed by the mutation name in parentheses, for example, *sup-5*(*e1464*) *III*. Strain names consist of 1 or 2 non-italicised upper case letters (indicating the laboratory of origin) followed by a number, for example, E1464. For most single mutants, strain and mutation names have the same letter and number but differ in typecase and by typeface, for example, the strain E1464 and the mutation *e1464*. Strains with multiple mutations are designated by the 1- or 2-letter laboratory prefix, a dash and a single upper case letter indicating the type of multiple mutant and number. Thus R is used to denote revertant strains, for example E-R215.

process. Especially useful would be a set of informational suppressors, which could then be used to study a wide variety of genes. Although suppressor mutations have been identified in *Drosophila melanogaster* (see ref. 20 for list), the mechanism involved is only partially understood²¹⁻²⁷.

For several years we have been studying mutants with abnormal muscle in the nematode *Caenorhabditis elegans* to identify genes involved in muscle assembly. Of the 13 genes now known to affect muscle structure (R.H.W., unpublished) the products of two are now known: *unc-15 I* specifies paramyosin²⁹ and *unc-54 I* specifies a major heavy chain of myosin³⁰⁻³³ (see footnote to Table 1 for nomenclature). At both loci we have isolated mutants that produce altered products, as well as null mutants in which none of the relevant polypeptide product can be detected²⁹⁻³². Mutants in these genes are particularly suitable for studies of suppression, for a few animals with even marginally enhanced motility can be easily detected against the background of the original paralysed mutant. Effects of suppression on muscle structure can be screened by polarised light microscopy, and biochemical analysis of the consequences of suppression can be readily carried out with the null mutants.

Reversion analysis of a large set of mutants with severely defective movement has already been carried out and some suppressor genes have been identified (refs 34, 35 and S.B., unpublished). In none of these cases is the molecular mechanism of compensation understood. In particular, Riddle and S.B.³⁴ have characterised a suppressor (*sup-3 V*) that partially reverses the phenotypic effects of many alleles of *unc-15*, *unc-54* and *unc-87 I*, another gene affecting muscle (R.H.W., unpublished). This and other evidence suggest that *sup-3* probably acts indirectly.

We report here the identification of a different suppressor (*sup-5 III*) isolated initially in reversion studies of E1214, a mutant of *unc-15* that is very paralysed, is fertile, but does not lay eggs, and contains no detectable paramyosin²⁹. The suppressor is allele-specific but not gene-restricted, suppressing

only some alleles in several different genes. It also has the important property of partially restoring paramyosin in animals homozygous for *e1214*. These observations suggest that *sup-5*(*e1464*) *III* might be an informational suppressor.

Isolation and preliminary genetic characterisation of the suppressed strain R215

Approximately 10⁵ E1214 animals, treated with 0.05 M ethylmethane sulphonate³⁵, were deposited on one side of 24 10-cm Petri plates. No full revertants appeared over the next three generations, but several plates contained animals with possibly improved movement. Most of these were later discarded but one strain that showed distinctively improved mobility was retained. Adults of this strain were larger than the E1214 strain and laid eggs, and the uniformity of the phenotype in the progeny indicated that the revertant was homozygous. F₂ progeny from a cross of the revertant with wild-type males showed that the revertant contained the original *e1214* mutation as well as an unlinked suppressor mutation (*C. elegans* normally reproduces as a self-fertilising hermaphrodite. It is diploid, with five autosomal linkage groups (I-V) and a sex linkage group (X). Males, which are hemizygous for the sex chromosome, are used to transfer genetic material. Heterozygous hermaphrodites can be allowed to self-fertilise to obtain homozygous animals.) From this cross a homozygous suppressed strain was re-isolated and propagated as the strain E-R213. To remove extraneous mutations, this strain was first crossed with *dpy-5 unc-15*(*e1214*)/+ + males to obtain a dumpy suppressed strain which was in turn crossed with *unc-15*(*e1214*)/+ + males to remove the dumpy marker. This yielded the strain E-R215 which was homozygous for both *e1214* and the suppressor allele, designated *e1464*. E-R215 grows better than E-R213, but motility and other characteristics do not differ appreciably.

Mapping the suppressor, *e1464*, in linkage group III

Location of the suppressor on linkage group III (LGIII) was determined initially from 2-factor crosses in a homozygous *e1214* background using general genetic methods described previously³⁶. An estimate of 7 map units between *dpy-18 III* and the suppressor was obtained, and results with *dpy-19 III*, *sma-2 III* and *lon-1 III* indicated that *e1464* was located less than 4 map units from these markers. As no previously mapped suppressors were in this region, *e1464* was assigned to a new gene *sup-5 III*. A strain containing the suppressor in a wild-type background, E1464, was obtained by elimination of the *lon-1* marker from a *lon-1* *+/+ e1464* animal.

A male stock of the E1464 strain was generated from spontaneously occurring males to facilitate the transfer of the mutation in further genetic manipulations. Several 3-factor crosses were carried out to position the suppressor more precisely (Table 1). In progeny of hermaphrodites of the genotype *sup-5(e1464)++/+unc-69 dpy-18* in cross 1, all the dumpy recombinants contained the suppressor, indicating that *sup-5(e1464)* lies to the left of *unc-69* or very close to it. From cross 2, only 12 of 20 dumpy recombinants contained the *trans* mutation *unc-69*, clearly placing *sup-5* to the left of *unc-69*. A third cross was carried out with *e1464 trans* to *dpy-18* and *unc-32*, a gene 3% to the left of *unc-69*. All dumpy recombinants contained the suppressor. A left-hand limit was established in cross 4, where *sup-5* was shown to lie to the right of *lon-1*. Crosses 3 and 4 place *sup-5* very close to *dpy-19* and *unc-32*, two genes that are themselves only 0.1% apart (Riddle, personal communication).

Phenotype of E1464

The E1464 strain moves normally, and its muscle structure as observed with polarised light microscopy²⁹ is indistinguishable from wild type. However, the strain shows some differences from wild type. Sterile animals are produced frequently, particularly when the strain is grown at 15 °C instead of room temperature (22 °C). Occasionally, dead early larvae and other abnormal forms have been noted. The generation time is increased to 7.5 d at 15 °C from the normal wild-type time of 5.5 d. Male stocks have been very difficult to maintain. Doubles of *e1464* with the linked markers *e364* and *e185* exhibit similar characteristics. This fact, together with the several crosses undergone in the construction of the E1464 strain makes it probable that these properties are due to the suppressor mutation itself.

Range of suppression by *e1464*

The *e1464* suppressor has been combined with a large number of different mutants of many genes to test for suppression. While mapping *e1464*, the phenotype of many mutants was found to be unaffected by *e1464*. These include the dumpy mutants *dpy-5(e61) I*, *dpy-10(e128) II*, *dpy-18(e189) III*, *dpy-13(e184) IV* and *dpy-11(e224) V*; the uncoordinated mutant *unc-32(e189) III*; the long mutant *lon-1(e185) III*; and the small mutant

sma-2(e502) III (ref. 36). However, *dpy-18(e364) III* gave anomalous results in initial mapping experiments, and subsequently it was shown that *e364* is suppressed by *e1464*. In strains homozygous for both *e364* and *e1464*, animals are nearly wild type in length and diameter when grown at 15 °C (Table 2).

Additional double mutants have been constructed to test the specificity of *e1464*-mediated suppression. In the *unc-15* complementation group, the mutant allele, *e73*, which results in a dysfunctional paramyosin, is not suppressed even though the null allele, *e1214*, is.

unc-54 mutants have also been studied, as the gene product is known to be a myosin heavy chain³⁰⁻³³. More than 30 independent mutations are available, and these have been classified into two groups; mutants of the larger class contain no detectable *unc-54* myosin, whereas those of the other contain approximately normal levels of gene product³¹. This second class is assumed to have altered product and, in fact, one mutant, E675,

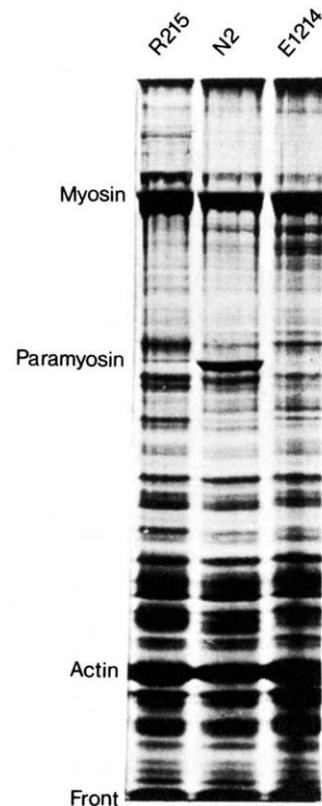


Fig. 1 Comparison of total SDS-soluble proteins from the nematode strains N2, E-R215 and E1214. Animals were grown in liquid culture, collected⁴⁰ and frozen²⁹ until use. Care was taken to obtain cultures of animals in similar larval stages to minimise possible developmental variations. 15 µl of a 1:7 (v/v) dilution of each sample was applied to an SDS-polyacrylamide gel³⁹. The gel was stained with Coomassie brilliant blue, destained and photographed.

Fig. 2 Electron micrographs of body wall muscle from the three strains, N2, E-R215 and E1214. Adult animals grown at 20 °C were prepared for microscopy as previously described²⁹, and transverse sections were viewed and photographed with an AEI EM6B electron microscope. Magnification 30,000×.

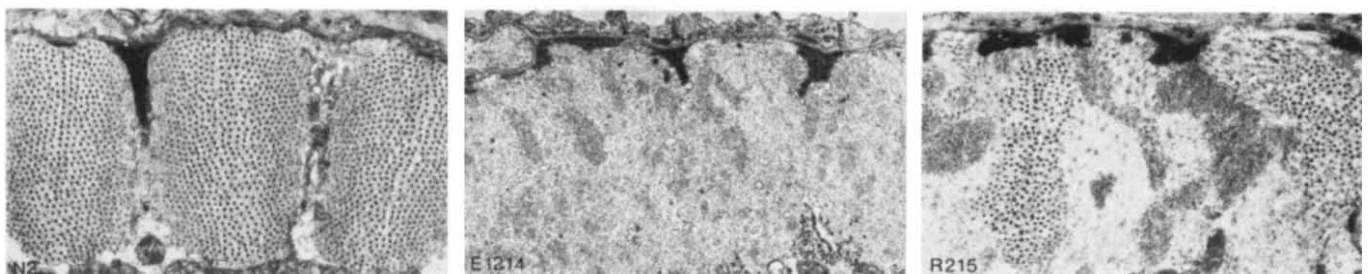


Table 2 Mutation suppression by *e1464*

Gene	Phenotype	Alleles suppressed by <i>e1464</i>	Alleles not suppressed by <i>e1464</i>
<i>unc-15 I</i>	Muscle defective (paramyosin)	<i>e1214</i>	<i>e73</i>
<i>unc-54 I</i>	Muscle defective (myosin heavy chain)	<i>e576, e1108</i> <i>e1021, e1008,</i> <i>e1419</i>	<i>e190, e569, e651,</i> <i>e675, e903,</i> <i>e1009, e1092,</i> <i>e1115, e1168, e1201,</i> <i>e1258, e1274, e1300,</i> <i>e1315, e1328, e1420,</i> <i>st1, st2, st3</i>
<i>unc-13 I</i>	Severely uncoordinated	<i>e312, e376,</i> <i>e450, e1091</i>	<i>e309, e323, e328,</i> <i>e973, e1038, e985,</i> <i>e387, e51, e158</i>
<i>dpy-18 III</i>	Dumpy	<i>e364</i>	<i>e499</i>
<i>cat-1 X</i>	Catecholamines lacking neuronal processes	<i>e1111</i>	NA
<i>nuc-1 X</i>	Lacks deoxyriboendonuclease	<i>e1392</i>	NA

Doubles of the suppressor with each of the above autosomal mutations were constructed by the elimination of markers closely linked either to the suppressor or to the mutant to be tested. For example, E1464 males were mated with the double mutant *unc-54*; *sma-2*, and six paralysed or slow F₂ progeny (those homozygous for *unc-54*) were selected. The progeny of each animal was inspected for suppressed animals. Those animals that did not segregate small progeny were assumed to be homozygous for *e1464*, as *sma-2* is less than 2% from *sup-5*. For sex-linked mutants, suppression was evaluated directly by observing the phenotype of males produced from a cross of mutant hermaphrodites with *e1464*/+ males. In the case of *nuc-1*(*e1392*), 63 of 148 male progeny were suppressed and with *cat-1*(*e1111*), 60 of 129 males had suppressed phenotypes, in good agreement with the predicted ratio. All the mutations listed, as well as those described in the text, were isolated after EMS treatment³⁶ except *e903* (X-ray mutagenesis)³⁰, *e1258* (DES mutagenesis) and *e1168* and *e1201* (ICR-22 mutagenesis) (Riddle, personal communication). Some of the mutants of *unc-15* (ref. 29), *unc-54* (refs 30, 31), *unc-13* (ref. 36), *nuc-1* (ref. 38), *dpy-18* (ref. 36), and *cat-1* (ref. 37) loci have been described previously. Only those which showed clear phenotypic suppression were scored as positive. NA, no other independent mutants are available for this locus.

has been shown to contain a small internal deletion in its heavy chain³². Of the 22 null mutants tested, five show clear phenotypic suppression. The suppressed animals move even as adults, lay eggs and attain a full adult size. Nonetheless, suppressed animals move much more slowly than wild type and are clearly distinguishable from them. Neither *e675* nor *e1274*, mutations resulting in altered proteins^{30,31}, is suppressed.

Several independent mutants that are extremely uncoordinated have been isolated in *unc-13 I*. Reversion studies had been previously carried out with mutants in *unc-13* and several alleles had been found to be suppressible by unlinked suppressors (S.B., unpublished). Of 13 alleles tested, four are suppressed by *e1464*. When both the suppressible *unc-13* allele and *e1464* are homozygous, animals have nearly wild-type movement; suppression is also apparent in animals homozygous for the *unc-13* allele but heterozygous for the suppressor. The suppressor mutation is semidominant. To test the effect of temperature on the suppressed strains, all the double strains have been grown at 15 °C and 25 °C. In all cases, the suppression is more pronounced in animals grown at 15 °C, consistent with the idea that the difference in suppression at the two temperatures is associated with the suppressor *per se*. These observations correlate with the increased sterility and slow growth noted at 15 °C in the E1464 strain.

Lastly, mutant alleles of two genes that have no relationship to muscle function or structure have also been tested. One, *cat-1*(*e1111*) X, alters the distribution of formaldehyde-induced fluorescence in catecholamine-containing neurones³⁷, and the other, *nuc-1*(*e1392*) X, results in the loss of detectable deoxyriboendonuclease activity³⁸. Phenotypically, the latter mutation results in the persistence of pyknotic nuclei of cells undergoing programmed cell death and in the presence of undigested DNA in the lumen of the digestive tract; these alterations are easily detected in Feulgen-stained animals. Clear suppression of both mutations was found when the suppressor was present only in a single dose (Table 2).

Mechanism of suppression

Because the protein products of *unc-15* and *unc-54* genes are known, suppressible mutations in these genes facilitate the determination of the molecular consequences of suppression. The E1214 strain contains essentially no paramyosin and virtually no normal thick filaments in the body wall muscle cells²⁹

(Figs 1, 2). When the total SDS-soluble proteins from the suppressed strain R215 are analysed by polyacrylamide gel electrophoresis, a faint band with a mobility similar to the paramyosin band from N2 is present, whereas among the E1214 proteins no such band is seen (Fig. 1). In addition, a protein has been purified from E-R215 showing mobility identical to wild-type paramyosin on SDS gels, but the yield of paramyosin is only about 10% of that obtained from wild type. Paracrystals have been prepared from R215 paramyosin and, when viewed with the electron microscope on negatively stained grids, they seem to be similar to paracrystals of wild-type paramyosin. Cyanilation cleavage of the E-R215 protein with 2-nitro-5-cyanobenzoic acid⁴¹ results in five fragments which have the same mobility on SDS gels as those obtained from wild-type paramyosin.

The structure of E1214 body wall muscles is also affected by the suppressor. By polarised light microscopy, E-R215 shows an increased birefringence, mostly localised in poorly defined, nearly longitudinal bands (presumably A-bands). In electron micrographs of transverse sections, E-R215 displays several of the ultrastructural features of E1214, but in addition, clusters of filaments having the diameter and appearance of wild-type thick filaments are present among abundant thin filaments. These thick filaments are much less numerous and less well ordered than in N2 (Fig. 2).

Preliminary analysis of the suppressed myosin mutant strain *unc-54*(*e576*); *sup-5*(*e1464*) indicates partial restoration of wild-type muscle structure. Myosin has been purified from the strain, but because myosin heavy chains in *C. elegans* are the products of more than one gene^{30,31}, it is more difficult to determine if the *unc-54* myosin is restored by the suppressor. However, cyanilation cleavage yields peptides that are unique products of the *unc-54* heavy chain³¹, and initial experiments indicate partial restoration of these peptides in *unc-54*(*e576*); *sup-5*(*e1464*). The large number of mutants in this gene may allow us to determine whether the restoration of gene product by the suppressor always correlates with phenotypic suppression.

Conclusion

Our experiments show that *e1464* suppresses mutants of several different genes, including two that code for muscle proteins, as well as others that are clearly not related to muscle. In the genes

in which several independent mutations are available, only a few of the mutants are suppressible; most are unaffected by *e1464*. The extent of phenotypic suppression is different in mutants of different genes: *unc-15* and *unc-54* mutants are weakly suppressed, whereas with *dpy-18*, *unc-13*, *nuc-1* and *cat-1* mutants a nearly wild-type phenotype is restored. These phenotypic differences probably reflect the functions of the gene products, as the weakly suppressed mutants involve proteins that are required stoichiometrically to form the muscle lattice. One of the well-suppressed mutants, *nuc-1*, affects an enzymatic function, and perhaps the others do also.

In E-R215, the suppressor partially restores the specific gene product (paramyosin) affected by *e1214*. This is also apparently the case with the suppressible myosin mutants. It is likely that in the double mutant *sup-5(e1464); nuc-1(e1392)*, endonuclease activity has been restored, but no direct tests have been carried out.

The suppressor is semidominant, or dose dependent. The heterozygous suppressor *e1464/+* is clearly effective in the strongly suppressed mutants, and a slight effect of the heterozygous suppressor can also be detected in *unc-15* and *unc-54* mutants. It should be feasible to demonstrate some restoration of paramyosin in *e1214/e1214; e1464/+* animals. The suppressor alone exerts a deleterious effect on the animal, particularly at low temperatures, where suppression of mutant phenotypes is greatest.

None of the *unc-15* and *unc-54* mutants which contain altered gene products is suppressed and the suppressor seems to act only on a subset of null mutants. We do not yet know why the null mutants are devoid of gene product. If ethylmethane sulphate produces principally G to A transitions in the nematode as it does in microorganisms⁴², then nonsense mutations producing premature chain termination are likely to account for most of the large set of null mutants. As yet, no truncated fragments have been identified in these mutants, but it is possible that they are degraded after formation or lost in purification. The mutant E675 does contain a shortened chain, but this results from a small internal deletion which leaves the C-terminus intact³². Thus the *e1464* mutation may be a specific nonsense suppressor analogous to those found in microorganisms, perhaps acting through an altered tRNA species. On the other hand, the suppressible mutations may affect some other step of information transfer and the suppressor may alter another component of the machinery. Further genetic and biochemical studies should reveal which of these possibilities is correct.

The mutant alleles used in the crosses were from the Cambridge collection. Additional alleles were from the St. Louis

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letters to nature

Contribution of intermediate luminosity X-ray galaxies to the background: Anon 0945–30

EVIDENCE that the galaxy A0945–30 is a new extragalactic X-ray source has been found from an analysis of rotating modulation collimator (RMC)¹ quick-look data collected from 53 orbits of the SAS 3 X-ray observatory during the period 26 February–2 March 1978. The exposure is approximately $7 \times 10^6 \text{ cm}^2 \text{ s}$. The galaxy lies within the error boxes quoted for 4U0945–30 and 2A0946–310 but is not one of the previously

suggested candidates. A faint star alongside the galaxy also lies in the 90% confidence X-ray source error circle (Fig. 1). The data are consistent with a point source². The flux from the source varied by a factor of about five during the period of our observation.

The SAS 3 RMC data alone do not allow a unique spectral fit. Fortunately, data from HEAO 1, (R. F. Mushotzky, personal communication) give a photon number index $\gamma = 1.8 \pm 0.1$ and a hydrogen column density of $N_H = 3 \pm 1 \times 10^{22} \text{ cm}^{-2}$ for a power law spectrum extending to about 35 keV. Using a 2 keV low-energy cutoff the SAS 3 data yield a slope identical with the HEAO 1 value. The X-ray flux density is $(4.5 \pm 0.6) \times 10^{-29} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$ at 10^{18} Hz (4.14 keV) and the 2–10 keV

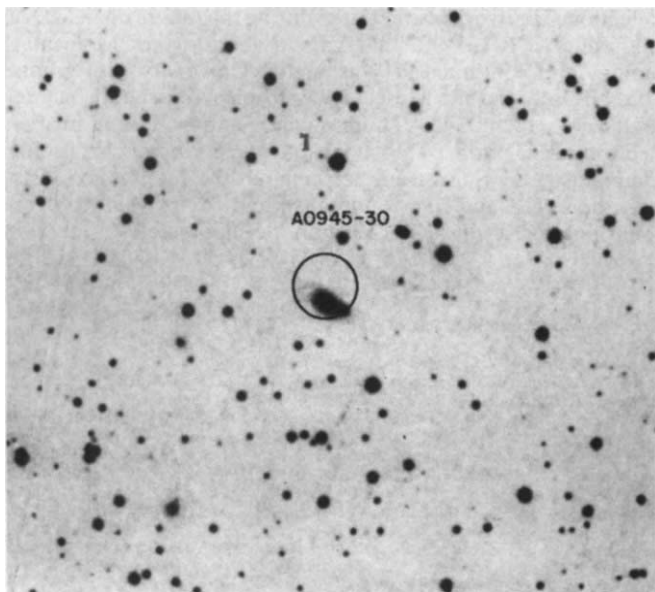


Fig. 1 The SAS 3 90% confidence error circle (radius = 35") superimposed on a photograph of A0945-30. The measured position of the X-ray source is $\alpha(1950) = 9\text{ h } 45\text{ min } 28.4\text{ s}$, $\delta(1950) = -30^\circ 42' 36''$ and the position of the galaxy is $\alpha(1950) = 9\text{ h } 45\text{ min } 28.43\text{ s} \pm 0.13\text{ s}$, $\delta(1950) = -30^\circ 42' 57.0 \pm 1.7''$.

X-ray flux is $(7.7 \pm 0.3) \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$. The measured heliocentric radial velocity is $2,498 \pm 15 \text{ km s}^{-1}$ and, with $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, the average X-ray luminosity is $\sim 2.2 \times 10^{43} \text{ erg s}^{-1}$.

The hydrogen column density inferred from the X-ray spectral cutoff indicates that the galaxy A0945-30 rather than the star is the X-ray source. In the source direction, the hydrogen column density in the Galaxy is at most $\sim 2 \times 10^{21} \text{ cm}^{-2}$ (refs 3, 4), nearly an order of magnitude less than the column density obtained by Mushotzky (personal communication).

Recent observations by Liller (personal communication) indicate that the galaxy is an early type spiral (possible an Sa) with a bright nucleus. Detailed optical observations of the galaxy and the star were made with the new digital spectrograph on the 1.5-m telescope at Mt Hopkins observatory. The star is an F dwarf with no indication of emission features. Plates in the Harvard collection covering this field show no evidence for long term variability of the star (L. Chaisson and J. P. Huchra, personal communication). Spectra of the galaxy in the red (4,500–7,000 Å) and blue (3,900–6,000 Å) show sharp, intermediate strength emission lines of [OIII], [NII] and H α together with strong Mg, Fe, Ca absorption features characteristic of a normal stellar continuum in the galactic nucleus (Fig. 2). The absorption-corrected absolute magnitude of the galaxy is ~ -20.0 . No significant H β emission is present. The other emission lines present are just resolved at the instrument resolution of $\sim 200 \text{ km s}^{-1}$ FWHM or 300 km s^{-1} FW0I (full width zero intensity) (Fig. 2). This width is considerably smaller than the widths of 1,800 and 1,100 km s^{-1} FW0I reported by Ward *et al.*⁵ for H β in the X-ray emitting galaxies NGC2992 (2A0943-140) and NGC7582 (2A2315-428). The width is also smaller than the $\sim 1,800 \text{ km s}^{-1}$ FW0I for H β (refs 6,7) in NGC 5506 (refs 8,9). NGC 2992, NGC 7582 and NGC 5506 are similar to weaker type 2 Seyferts. The emission line equivalent widths are much smaller in A0945-30 than in NGC 5506 (20 Å as opposed to 200 Å for $\lambda 5007$ Å) which could be due to larger extinction of the nuclear source; the correction for dilution by the galactic continuum in our long slit ($4'' \times 25''$) observation only increases the emission line equivalent widths for A0945-30 by a factor of ~ 2 . The excitation of both galaxies is quite high with $I(\lambda 5007)/I(\text{H}\beta) \sim 10$ in NGC5506 and ≥ 20 in A0945-30.

Our optical observations of the galaxy A0945-30 strengthen the case for its identification with the X-ray source. Previously, it would have been unreasonable to identify A0945-30 with 4U0945-30 or 2A0946-310 because of the large area error boxes associated with these non-modulation collimator observations. Identifications would be made only when well-known objects which are members of established classes of X-ray emitters were in the error box.

A0945-30, NGC5506, NGC2992 and NGC7582 are members of an X-ray optical luminosity class characterised by X-ray luminosities of $\sim 10^{43} \text{ erg s}^{-1}$ and with $-20.5 \leq M_B \leq -19.0$. The number density of all galaxies in this absolute magnitude range is $\sim 10^{-2} \text{ Mpc}^{-3}$ (ref. 10). The $10^{43} \text{ erg s}^{-1}$ X-ray galaxies could, therefore, be numerous and it is interesting to speculate on the possibly significant contribution of

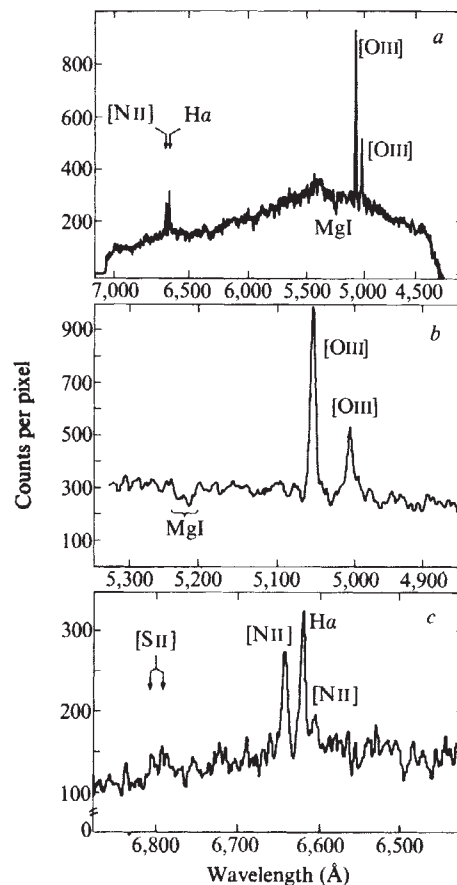


Fig. 2 a, A spectrum of A0945-30 obtained during a 15-min integration with the new digital spectrograph on the 1.5 m Mt Hopkins telescope. b and c, enlarged sections of the spectrum showing prominent emission and absorption features. The data have been smoothed to the resolution limit of about 5 Å. The detector has a resolution of ~ 1.8 Å per pixel.

objects in this range of the X-ray-optical luminosity function to the X-ray background.

In the 1–20 keV range the X-ray background intensity $I(E) = 8.5E^{-0.5 \pm 0.2} \text{ keV (keV}^{-1} \text{ cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1})$ (ref. 11). If the emissivity of extragalactic X-ray sources per unit co-moving coordinate volume is independent of cosmological epoch, then already-known classes of discrete sources (Seyferts, quasars, clusters of galaxies) can account for at most 40% of the X-ray background¹². The X-ray luminosity density required to account for the remaining 60% of the background intensity is $\sim 10^{39} \text{ erg s}^{-1} \text{ Mpc}^{-3}$. The required number density of $10^{43} \text{ erg s}^{-1}$ sources is 10^{-4} Mpc^{-3} . To account for the background, only 1% of galaxies in the magnitude range $-20.5 \leq M_B \leq -19.0$ need be $10^{43} \text{ erg s}^{-1}$ X-ray emitters.

The background spectrum ($\alpha = 0.5 \pm 0.2$) in the 1–20 keV range could be produced by discrete sources with a range of power law spectra. One consistent model for the distribution of spectral indices is a gaussian with mean spectral index $\bar{\alpha} = 0.5$ and dispersion $\Delta\alpha = 0.4$ (ref. 13). The spectra of A0945–30 ($\alpha = 0.8 \pm 0.1$) NGC5506 ($\alpha = 0.3 \pm 0.3$) and NGC7582 ($\alpha = 0.3 \pm 0.4$) (R. Mushotzky, personal communication) are all in the expected range.

Uhuru observations reveal fractional fluctuations in the 2–10 keV background intensity of $\delta I/I = 3.6 \pm 0.5\%$ for an effective solid angle of $\Omega = 0.004$ ster (ref. 11). If the 10^{43} erg s $^{-1}$ sources are randomly distributed with mean density 10^{-4} Mpc $^{-3}$, the expected $\delta I/I \sim 2\%$, consistent with the observational limit. If these X-ray emitting galaxies are clustered in the same way as the general galaxy population¹⁴, the fluctuations are increased by at most a factor of 2.

The X-ray luminosities of individual clusters of galaxies ($\sim 10^{44}$ erg s $^{-1}$) (ref. 12) provide another constraint on the acceptable fraction of galaxies like A0945–30. Photometry of four X-ray clusters^{15,16} indicates that the expected mean number of 10^{43} erg s $^{-1}$ X-ray galaxies is at most one per cluster.

The SAS 3 RMC survey covers $\sim 5\%$ of the sky to a limiting flux of $\sim 2.5 \times 10^{-11}$ erg s $^{-1}$ cm $^{-2}$. A 10^{43} erg s $^{-1}$ source could be detected at a distance of ~ 60 Mpc. If the mean density of sources is 10^{-4} Mpc $^{-3}$ the survey should have detected 4–6 sources in the $1\text{--}2 \times 10^{43}$ erg s $^{-1}$ range. The detection of three sources (A0945–30, NGC5506, and NGC7582) indicates that these galaxies may be sufficiently numerous to account for $\sim 50\%$ of the X-ray background. The number of sources required is also consistent with the number of unidentified sources in the high latitude ($|b| > 20^\circ$) Uhuru (ref. 17 and C. Jones, personal communication) and Ariel¹⁸ surveys which have limiting 2–10 keV sensitivities of 5×10^{-11} erg cm $^{-2}$ s $^{-1}$ and 6.1×10^{-11} erg cm $^{-2}$ s $^{-1}$ respectively. We expect Uhuru detection of 20–30 of these $1\text{--}2 \times 10^{43}$ erg s $^{-1}$ sources and Ariel detection of 15–20 such sources. To produce $\sim 50\%$ of the X-ray background intensity we require less than half of the completely unidentified high latitude sources in either survey to lie in this intermediate X-ray-optical luminosity range.

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SS Cygni as a hard X-ray source identified with the scanning modulation collimator on HEAO 1

X-RAY emission from the dwarf nova SS Cygni was first detected at soft ($\sim \frac{1}{4}$ keV) X-ray wavelengths when the source was in an optical high state^{1–3}. More recently, ANS satellite observers⁴ have reported the detection of soft X-ray emission when SS Cygni was in an optical low state. These latter observations show the flux of the soft X-ray source to be about an order of magnitude less than the values when in the optical high state. The soft X-ray positional uncertainty is ~ 0.2 deg 2 . The correlation between optical and soft X-ray behaviour convincingly identifies SS Cygni as a soft X-ray source. Hard (> 1 keV) X-ray emission from a $15' \times 1.5^\circ$ area containing SS Cygni was also reported by the ANS observers during the optical low state and identified with SS Cygni⁴. A more accurate position (0.03 deg 2) has been reported by Ariel 5 observers⁵. We report here a 0.4 arc min 2 measurement of the position of the X-ray source with the HEAO 1 modulation collimator experiment which leads to an unequivocal identification of SS Cygni as an X-ray emitter in the 2–10 keV energy range. We have also searched the Uhuru data to investigate the historical light curve in hard X rays.

The +Y axis instruments of the HEAO 1 satellite were pointed at SS Cygni for two orbits on 16 December 1977, when it was in an optical low state. The modulation collimator (MC) experiment data were binned⁶ about the position of SS Cygni. We used only one orbit of data (~ 1 h of observing time) because of problems in the determination of the aspect for the second orbit. Moreover, we rejected an additional 18% of the data when there were rapid changes in the background level.

The ANS and Ariel 5 error boxes were used to reduce the multiplicity in our positional determination. Five MC diamonds (Fig. 1) are within the ANS error box and only one, which includes SS Cygni (Fig. 2), is in the Ariel 5 error box. The area within this diamond is 0.4 arc min 2 . This is a more than 200-fold improvement with respect to previous results^{4,5}, and definitively confirms that SS Cygni is a hard X-ray emitter.

The MC flux (2–6 keV) is $(1.7 \pm 0.5) \times 10^{-10}$ erg cm $^{-2}$ s $^{-1}$ with an additional 30% systematic uncertainty. The fit of the three A-3 spectral channels to a bremsstrahlung spectrum with Gaunt factor and low energy cut-off gives an equivalent temperature $kT > 10$ keV and a low energy cut-off $< 0.2 \times 10^{22}$ N $_H$ cm $^{-2}$.

Oscillations of small amplitude (< 0.002 mag) with a period of 9.75 ± 0.03 s have been reported in the optical flux of SS Cygni during an optical maximum (Robinson, personal communication). We searched our data for periodicities, by folding according to different trial periods and calculating the χ^2 value for the hypothesis of a constant flux. We did not find any significant enhancement in χ^2 that could be attributed to the presence of pulsations for periods in the range 5–20 s. The 3σ upper limit for the pulsed fraction is $\sim 13\%$ for periods consistent with that of the optical pulsations.

We have investigated the historical light curve in hard X rays by analysing all the relevant Uhuru data. SS Cygni was in the Uhuru field of view ($0.5^\circ \times 5^\circ$ FWHM) and not confused with nearby sources in 12 d of observations. These data are scattered over 1.5 yr, but they are always coincident with an optical low state. In two instances there was marginal evidence for source detection in the 2–6 keV band: at an intensity of $(4.4 \pm 2.7) \times 10^{-11}$ erg cm $^{-2}$ s $^{-1}$ on 20 June 1971 and at $(9.0 \pm 3.2) \times 10^{-11}$ erg cm $^{-2}$ s $^{-1}$ the following day. These are of the same order as the ANS and HEAO 1 fluxes. Ariel 5 observed SS Cygni for a period of 120 d (ref. 5) including both low and high optical states. While the equivalent 2–6 keV flux during low states is highly variable between 7 and 30×10^{-11} erg cm $^{-2}$ s $^{-1}$, in the optical high state there is only an upper limit (2σ) of 6×10^{-11} erg cm $^{-2}$ s $^{-1}$.

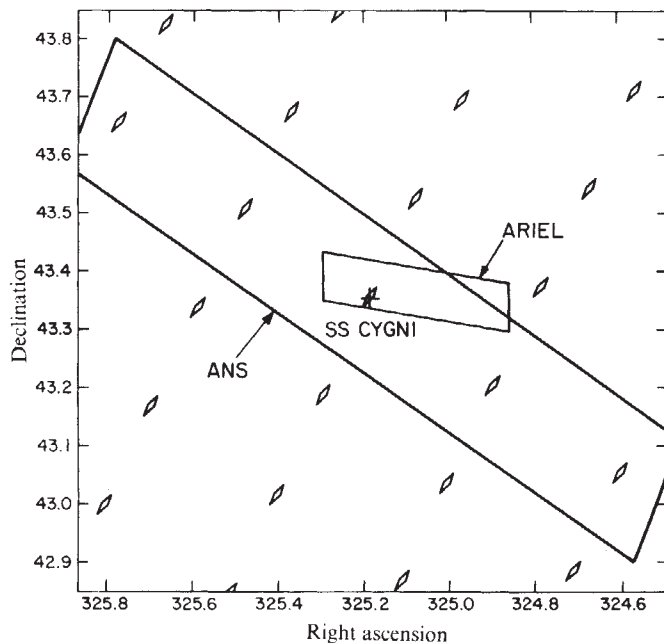
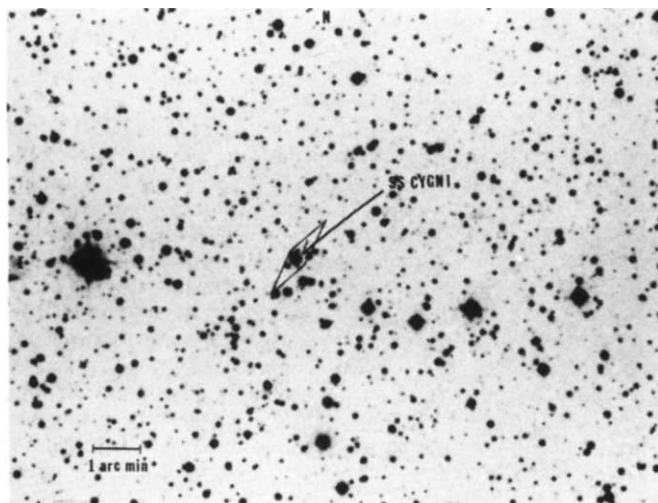


Fig. 1 One of the multiple MC positions (diamonds) falls within the previous location uncertainty measured by ANS (ref. 4) and Ariel 5 (ref. 5). This region has an area of 0.4 arc min^2 , and contains SS Cygni near its centre.

The X-ray luminosity of SS Cygni in the 2–10 keV energy range is then of the order of $10^{33} \text{ erg s}^{-1}$ (for a distance of $\sim 200 \text{ pc}$) in the optical low state and probably at least an order of magnitude less in the high state. In contrast, the soft X-ray luminosity increases by an order of magnitude between the optical low and high states: from 2 to $30 \times 10^{31} \text{ erg s}^{-1}$ respectively. The average spectral behaviour (0.25–10 keV) of SS Cygni thus shows two distinct states correlated with the optical behaviour: the effective spectrum seems to soften during the optical high states.

SS Cygni is the brightest of the dwarf novae, with a visual magnitude ranging between 12.1 at minimum and 8.1 at maximum (see reviews by Glasby⁸ and Robinson⁹). Its light curve shows outbursts that repeat with an average period of 50.6 d, but with a range of periods between 33 and 71 d. It is a spectroscopic binary system composed of a normal late type star orbiting about a white dwarf with a period of 6 h 38 min. The masses of both

Fig. 2 Enlargement of the POSS region containing SS Cygni, showing the MC location precision.



components are estimated to be in the range $0.8\text{--}1.0 M_{\odot}$. The continuum emission seems to be yellow at the minimum of the light curve and blue-violet at the maximum. The spectrum is characterised by hydrogen emission lines at the beginning of the rise to the optical maximum and hydrogen absorption lines that become broader while the luminosity reaches the maximum.

It is believed (see Paczynski in Preprint No. 73 of the Polish Academy of Sciences) that the white dwarf and its accretion disk are the source of the optical and ultraviolet as well as the soft X-ray emission in the dwarf novae. There is observational evidence that links the emission and absorption lines to the accretion disk⁹. The soft X-ray flux could either originate from the boundary layer of the disk and the white dwarf¹⁰ or from the surface of the disk which could behave as a corona¹¹ (B. Paczynski, personal communication). The limited information on the hard X-ray emission does not permit us to discriminate between these two hypotheses. A coronal model can explain the inverse correlation of the soft and hard X rays which is also true if accretion on to the white dwarf is the production mechanism of the hard X rays. In the coronal hypothesis the soft and hard X-ray fluxes both originate in the accretion disk, due to the interaction of magnetic fields and plasma, analogous to the solar corona. As discussed by Galeev *et al.*¹¹ The hard X-ray flux can be produced in magnetically confined plasma (loops arising from the disk surface) while the soft X-ray flux arises from regions of less intense magnetic activity. The increase in the soft X-ray flux, which could result from an increase in the accretion rate during the optical maximum, would cause a decrease in the hard X-ray flux by comptonisation. If accretion on to the white dwarf is the production mechanism of the hard X rays, as in the model of Fabian *et al.*¹², the anticorrelation of the soft and hard fluxes could be explained by a variation in the optical thickness of the region emitting hard X rays. This might result from the increased density of the accreting gas during the optical outbursts. The soft X-ray flux, which originates further away from the surface of the white dwarf, is not likely to be affected in the same sense. Instead, the increase in the temperature of the accretion disk during optical maximum could enhance the soft flux. The accretion mechanism for SS Cygni is discussed in detail by Ricketts *et al.*⁵. The lack of a cut-off at the low energies in the hard X-ray spectrum of SS Cygni is consistent with the $\sim 35^\circ$ inclination of the orbit of the system and with accretion from a disk.

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Long-term variability of galactic X-ray sources

THE long-term (days to months) variability of 32 persistent galactic X-ray sources brighter than Cas A in the energy range 3–10 keV is discussed here, using data from the MIT experiment on the OSO 7 satellite¹. For each X-ray source, we compiled a list of counting rates for all observations made during the first 600 d of operation of the experiment² (typically six or seven observations for each source). The results in these lists show the well-known high variability of the massive early-type X-ray binaries. Of special interest, however, are the variability characteristics of the sources associated with old populations. These include several near the galactic centre which are generally referred to as 'bulge' sources, and several which are known to lie in globular clusters. They share certain similar X-ray characteristics, namely, spectra which are markedly softer than those of massive binaries and an apparent absence of pulsations and eclipses^{3–6}. Because of their similarities, it has been suggested that they are all systems of the same kind, such as neutron stars in low mass binary systems^{7,8}, or the products of core collapse in globular clusters⁹. However, our results show that the long-term variability of the bulge sources is generally much less than that of the sources which lie in globular clusters. This suggests that there is a substantial difference between the bulge and globular cluster sources either in their nature or, at least, in certain critical properties of their components such as the masses of the nuclear-burning companions which may supply the material for accretion by compact objects in binary systems.

Only those sources at galactic latitudes in the range $|b^{\text{II}}| < 10^\circ$ were included in our study. An average counting rate was derived for each observation of each source. A typical observation lasted several days and was separated from other observations of the same source by periods ranging from a few days to a few months. The duration of each observation was sufficiently long to average out the effects of eclipses of the X-ray binaries. We considered only data from our 3–10-keV detectors because they were largely uncontaminated by solar X rays or trapped particles. For this study we selected a subset of sources that were sufficiently bright so that variations of intensity $> 10\%$ would be discernible. Specifically, we chose those sources for which, during at least one observation, a counting rate greater than two standard deviations above 2.5 counts s^{-1} was measured. This intensity is roughly that of the Perseus Cluster and the Cas A X-ray sources, generally believed to be nonvariable and which could, therefore, be used as standards (see ref. 2 for details).

For each source we computed a parameter which measures the variability from observation to observation. This parameter is defined by

$$\xi = \alpha / \bar{x} \quad \text{where} \quad \bar{x} = \frac{\sum_i (x_i / \sigma_i^2)}{\sum_i (1 / \sigma_i^2)}$$

and

$$\alpha = \left(\frac{\sum_i (x_i - \bar{x})^2 / \sigma_i^2}{\sum_i (1 / \sigma_i^2)} \right)^{1/2}$$

where x_i is the counting rate for each observation and σ_i is the

statistical uncertainty associated with each counting rate. The summations are carried out over all the observations of the sources in question. $\bar{x}$ is the mean counting rate and α is the r.m.s. deviation of the counting rates from this mean. (Each intensity measurement is weighted by its errors, see ref. 10.) ξ , defined as $\alpha / \bar{x}$, represents a measure of the fractional fluctuation of the x_i about $\bar{x}$. (We have not considered, in detail, the intensity distribution functions of all the sources.)

We also computed a parameter defined by

$$\Delta\xi = \bar{\sigma} / \bar{x} \quad \text{where} \quad \bar{\sigma} = \frac{\sum_i 1 / \sigma_i}{\sum_i 1 / \sigma_i^2}$$

$\bar{\sigma}$ is taken to be the magnitude of typical errors in the intensity measurements. The parameters of all the sources were computed for the two sets of data collected by the detectors behind the 1° and 3° collimators separately¹.

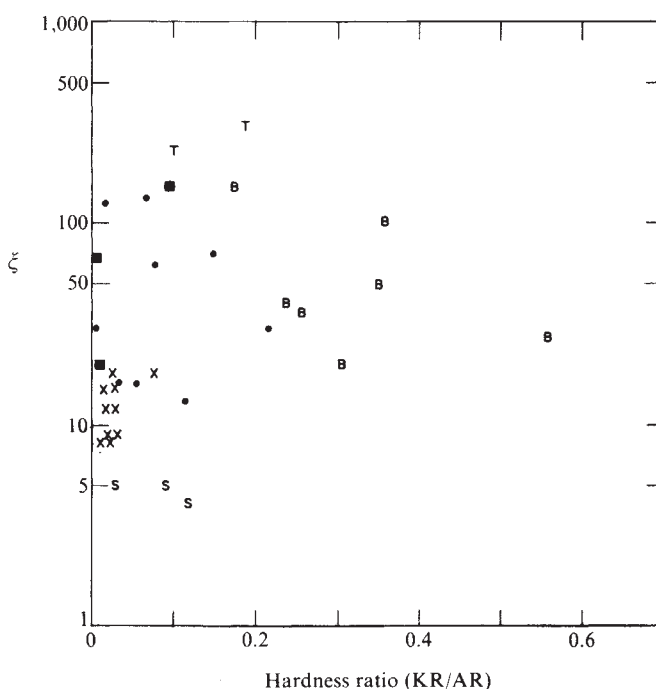


Fig. 1 The variability measure ξ is plotted against the spectral hardness (KR/AR) for the sources listed in Table 1. Standards (S) are the Crab Nebula, the Perseus Cluster and Cas A; GB (x), galactic bulge sources; GC (■), sources in globular clusters; B, binary X-ray sources; and T, transient X-ray sources. Those sources in Table 1 that are not yet known to be one of the above classes are plotted as dots.

Table 1 summarises the results on all the sources that satisfy the above selection criteria. The value of ξ listed for a source is the higher one from the two data sets. If the values of ξ measured for a source are comparable in the two sets, the one with a smaller $\Delta\xi$ is listed. Also listed are the value of $\Delta\xi$ and the number of observations (ν) for that source. We emphasise that $\Delta\xi$ should not be viewed as an error for ξ but merely as an indication of the counting statistics in the various measurements. For an X-ray source with constant intensity, the expected value of ξ is $\sim \Delta\xi$. The sources in Table 1 are ranked in increasing ξ ; also included are the Crab Nebula, the Perseus Cluster and Cas A which are generally believed not to vary significantly on

Table 1 Variability characteristic of galactic X-ray sources

X-ray source	ξ ($\times 100$)	$\Delta\xi$ ($\times 100$)	ν	Detector	Class*	Comment
3U0531+21	4	4	5	3		Crab Nebula
3U0316+41	5	6	6	3		Perseus cluster
3U2321+58	5	5	5	3		Cas A
3U1744-26	8	7	6	3	GB	GX3+1
3U1758-20	8	7	6	3	GB	GX9+1
3U1758-25	9	4	6	3	GB	GX5-1
3U1728-16	9	1	7	3	GB	GX9+9
3U1811-17	12	8	6	3	GB	GX13+1
3U1813-14	12	1	6	3	GB	GX17+2
3U1624-49	13	21	11	1		$l'' = 335^\circ$, $b'' = 0^\circ$
3U1735-44	15	9	10	1	GB	Burst source
3U1642-45	15	6	7	3	GB	GX340+0
3U1636-53	16	6	8	1		Burst source
3U1837+04	16	5	5	3		Ser X-1, burst source
3U1755-33	18	9	6	3	GB	
MX1709-40	18	8	9	1	GB	
3U1746-37	20	13	6	3	GC	NGC 6441
3U1956+35	20	10	4	1	BI	Cyg X-1
3U0900-40	27	9	4	3	BI	Vela X-1
MX1608-52	30	13	11	1		
3U2142+38	31	1	3	3	B	Cyg X-2
3U1728-24	36	7	7	1	B	GX1+4, pulsar
3U2030+40	40	7	5	3	B	Cyg X-3
3U1223-62	50	25	5	1	BI	GX301-2, pulsar
3U1820-30	63	13	6	1	GC	NGC 6624, burst source
3U1658-48	67	7	11	1		GX339-4
3U1320-61	70	30	5	3		
3U1258-61	103	14	5	3		GX304-1, pulsar
3U1705-44	127	38	7	3		Burst source (?)
3U1516-56	132	15	2	3	B	Cir X-1
MX1746-20	152	12	4	1	GC	NGC 6440
3U1118-60	153	24	4	1	BI	Cen X-3
3U1908+00	218	6	5	3		Aql X-1, transient (?)
3U1630-47	231	50	8	1		Recurrent transient
3U1543-47	304	51	12	1		Transient

* GB, galactic bulge; GC, globular cluster; B, binaries (?); BI, binaries associated with young massive primaries.

the time scale under study. The observed values of ξ and $\Delta\xi$ for these sources indicate that they are nonvariable (within an uncertainty $\Delta\xi$). As systematic errors affect the results for weak sources more than those for strong ones, we limited our study to sources brighter than Cas A.

Figure 1 shows the relationship between long-term variability and spectral hardness as characterised by the ratio of the counting rate in the 15–40-keV channel to that in the 3–10-keV channel². As expected, Table 1 and Fig. 1 show the high variability of young massive binary sources (BI). In contrast, the bulge sources (GB) are, for the most part, either steady or only slightly variable. The six least variable sources (excluding the 'standard' sources) are GB sources. The absence of long-term variability is, therefore, a distinguishing characteristic of the bulge sources.

The sources in globular clusters (GC), on the other hand, vary in about the same degree as the young binary sources. Although the sample of GC sources is small, two of the three in our list are much more variable than any of the GB sources. Three other globular cluster sources, NGC1851, NGC6712 and M15, which failed to make our list because of their low intensities, are also known to be highly variable¹¹.

Sources of type I X-ray bursts are found both inside and outside globular clusters (for example, the burst source associated with the globular cluster NGC6624 (refs 12, 13) and MXB1735-44 which is identified with an isolated faint blue star¹⁴⁻¹⁶; for detailed properties of X-ray burst sources, see ref. 17). It seems likely that the globular cluster sources and all the type I burst sources, both inside and outside globular clusters, form a class of similar objects (F.L. and G.C. in preparation).

They could, for example, be non-magnetised neutron stars in low-mass binary systems (see ref. 18 for a model). If the bulge sources near the galactic centre are, as we conjecture, of the same nature, then the systematic difference in the long-term variability must be attributed to differences in some critical parameter(s) affecting the mass transfer process. One such parameter, for example, may be the mass of the nuclear burning companion. The typical mass of the nuclear burning companion of an X-ray binary in the core of a globular cluster would be $\sim 0.5 M_\odot$. Low-mass X-ray binaries formed outside globular clusters such as by the evolution of cataclysmic variables¹⁹ may have nuclear burning companions with masses of $\sim 1.0 M_\odot$.

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Definition of a black hole

TIPLER has given a new definition for a black hole¹. These objects had previously been properly defined only in asymptotically flat spacetimes², but it seems unlikely that our Universe is one of these. One of the well known properties of a black hole, proved in asymptotically flat spacetimes, is that its 'surface area' can never decrease. By means of this new definition Tipler has shown that this area theorem may well not be true in our Universe. However, Tipler's definition is complicated, and it is not obvious that it corresponds to our usual concept of a black hole. I show here that it is equivalent to a definition that is both simpler and more clearly a formalisation of what we mean by a black hole.

I use the notation of refs 1 and 2. Let T be the set of points of a stably causal spacetime which belong to non-cosmological trapped surfaces. Tipler defines a black hole as the closure of the topologically smallest future set I^+ such that I^+ contains T , and the boundary of I^+ is generated by (future inextendible) null geodesic segments which are boundary generators of TIPS.

We would like a black hole to contain T . It should not be possible to escape from a black hole, so the hole should also contain $I^+(T)$. Furthermore, any points from which it is impossible to avoid falling into a black hole might as well be said to be within that hole. That is, a black hole should be the closure of $B = D^-(I^+(T))$; I shall show that B satisfies the conditions set out by Tipler for I^+ .

B is a future set: suppose $x \in B$ and $y \in I^+(x)$. There is a future directed timelike curve from x to y . If it meets $I^+(T)$ then $y \in I^+(T)$, so $y \in B$. If it does not meet $I^+(T)$, it must do so when extended along any future directed non-spacelike curve α from y as $x \in D^-(I^+(T))$. Therefore, any such α meets $I^+(T)$, so $y \in B$.

The boundary of B is generated by null geodesic segments which are boundary generators of TIPS: let x be on the boundary of B . Let (x_i) be a sequence of points lying to the past of x , not in B , with x as their limit point. There is a future inextendible non-spacelike curve γ_i from each x_i that does not enter $I^+(T)$. By proposition 6.2.1. of ref. 2 there is a future inextendible non-spacelike curve γ from x which is a limit curve for the γ_i .

As $\bar{B}$ is a closed future set γ can never leave it. As no γ_i can enter B , γ cannot enter the interior of B . But $I^+(\gamma) \subset B$, so γ cannot enter its own future. Therefore, it must be a null geodesic.

$\bar{I}(\gamma)$ is a TIP (see ref. 3). As γ cannot enter its own future it cannot enter its own past, $I^-(\gamma)$. Therefore, it is a boundary generator of $I^-(\gamma)$.

It remains to be proved that B is the smallest such I^+ . It has been shown that from any point on the boundary of B there is a future directed null geodesic γ that does not enter the interior of B , so cannot enter $I^+(T)$. Therefore, the boundary of B is not part of B , and B is open.

Suppose there is an I^+ that is contained in B but not equal to it. We can then find a point p which is in B but not in I^+ . Let α be a future directed timelike line from p . It must meet $I^+(T)$, and hence must enter I^+ . Let it cross the boundary of I^+ at y . There is a future directed null geodesic generator γ of the boundary of I^+ from y . It is future inextendible, and cannot enter $I^+(T)$ as otherwise it would be in the interior of I^+ . Thus, by proceeding from p along α to y then along γ , we can construct a future inextendible non-spacelike curve from p which does not meet $I^+(T)$. This contradicts $p \in B$.

Therefore B is the smallest such I^+ , and the black hole defined by Tipler is exactly $\bar{D}^-(I^+(T))$.

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Neutrinos from binary pulsars

PULSARS are believed to produce high energy particles. If high energy protons produced by the pulsar strike a target, say a binary companion, high energy neutrinos are produced in the collisions. At the same time, schemes for detecting high energy neutrinos produced in nature are currently being considered (refs 1–4 and K. Lande, personal communication). Here we point out that binary systems containing moderately young pulsars (ages $T < 10^4$ yr, that is comparable to NP0532) may emit high energy neutrinos ($10^{12} < E_\nu < 10^{14}$ eV) at detectable levels. Such pulsars are assumed to have total luminosities of the order 10^{38} erg s⁻¹. Discussion of feasible neutrino telescopes (such as the deep underwater muon and neutrino detector, DUMAND^{1–4}) suggests that fluxes from point sources as small as 10^{-2} – 1 eV cm⁻² s⁻¹ in high energy neutrinos may eventually be detectable. Neutrino detectors on a more modest scale, such as that of Lande (personal communication) will be operating within 1–3 yr, and have detection thresholds of 10^5 eV cm⁻² s⁻¹. Thus, a pulsar emitting 3×10^{38} erg s⁻¹ in high energy neutrinos at 3 kpc would be detectable even with Lande's detector, and the DUMAND detector may be able to detect sources that are weaker by three to five orders of magnitude.

We assume that at least some of the pulsars emit a fair fraction ($\approx 1\%$) of their energy in the form of high energy protons ($10^{13} < E_p < 10^{16}$ eV). This energy range is consistent with the upper limit⁵ of the potential near the star

$$V_{\max} = 10^{16}(B/10^{12} \text{ G})(\Omega/200 \text{ s}^{-1})^2 V \quad (1)$$

where B is the magnitude of the magnetic field at the stellar surface, and Ω is the frequency of rotation. That young pulsars can produce very energetic particles is implied by the very energetic ($E_\nu \approx 10^{12}$ eV) pulsed γ rays⁶ emitted by NP0532. The energy in the protons is converted efficiently to high energy neutrinos if either the protons traverse more than ~ 50 g of material, or they traverse a column density of 10^{28} photons cm⁻² in photons that are not too far above the pion production threshold in the rest frame of the protons. In each case, the protons are stopped by pion and kaon producing processes, and the charged mesons that are produced decay into three neutrinos and an electron. Roughly half the energy in the original proton goes into high energy neutrinos, which typically have energies of the order $10^{-2}E_p$ to $10^{-1}E_p$ (ref. 3).

There are several plausible ways in which the high energy protons might encounter enough target material to convert 1% to 50% of their energy to neutrinos.

If the companion star fills its Roche lobe, it could occupy nearly half the sky as seen from the pulsar, thus blocking a proportionate fraction of the high energy protons. Moreover, the overflowing material may completely engulf the pulsar.

Accreting matter can pile up at the magnetopause (which in the case of a pulsar would be made up of very low frequency electromagnetic Alfvén waves). It has been suggested^{7,8} that such a pile up occurs in several intermediate and rapidly rotating binary X-ray sources.

Or, the accreting matter might settle into a disk much smaller than the binary orbit, and much larger than the light cylinder. Such a disk could have a random orientation relative to the rotation axis of the pulsar, and could block a fair fraction of the protons leaving along open field lines. Moreover, as the disk may be substantially heated by such protons, it is likely to have a corona that completely engulfs the pulsar.

Besides being a target itself, matter near the pulsar can scatter and trap photons that also may serve as a target. To fix the ideas, suppose that 10^{38} erg s⁻¹ of X rays are emitted by the pulsar and diffuse away from it through surrounding matter. Assume the scale height of the photons is 3×10^8 cm and that their mean free path is λ ($\lambda < 3 \times 10^8$ cm). Then the density of photons at the scale of 3×10^8 cm is $3 \times 10^{19} (\lambda/3 \times 10^8 \text{ cm})^{-1}$, enough to stop all the protons by photopion production.

Finally, we discuss stellar winds. Suppose the pulsar's companion is an OB supergiant. Motivated by standard models of binary X-ray sources⁹, we assume a mass loss rate $\dot{M} = 10^{-5} M_{\odot} \text{ yr}^{-1}$, a wind velocity near the pulsar of $V = 300 \text{ km s}^{-1}$, and a binary orbit radius of 3×10^{11} cm. Then the column density of the wind surrounding the pulsar is of the order of (depending on direction) $1\text{--}10 \text{ g cm}^{-2}$, thereby providing a significant target. Reasonable parameters for red giants, $\dot{M} = 3 \times 10^6 M_{\odot} \text{ yr}^{-1}$, $v = 10 \text{ km s}^{-1}$, $R = 3 \times 10^{13}$ cm, yield a comparable column density. Pulsar emission impinging on the companion could stimulate a wind regardless of its type as has been suggested¹⁰ for the case of Cyg X3.

Assuming a pulsar formation rate of one every 10 yr (ref. 11), there ought to be $\sim 10^3$ moderately young pulsars in the Galaxy at present and more if pencil beaming effects allow a selection efficiency of only a few per cent. Older pulsars, though less powerful, are more numerous, and may also be detectable in neutrinos. Direct evidence that neutron stars can form without disrupting the binary system (such as binary X-ray sources) combined with the observation that a significant fraction of all stars (at least $\sim 50\%$ (ref. 13) perhaps most¹⁴) are in binaries allows the hypothesis that a significant fraction of these young pulsars are in binaries.

One route for binary pulsar formation particularly favourable to neutrino production is that a helium star or white dwarf, accreting the Roche lobe overflow from a red giant companion, becomes massive enough to explode, leaving a pulsar. (An explosion is not necessary, the pulsar may form quietly¹².) Just after the explosion, the companion to the young pulsar is automatically in the stage where its extended envelope and overflow play a significant role as target material.

It is difficult to predict the *a priori* probability of detecting, by conventional means, any pulsars that might exist in binary systems. First, there are beaming effects. Moreover, if there is some mechanism that accelerates pulsars to $\sim 250 \text{ km s}^{-1}$ at their inception (refs 15, 16 and refs therein), only the ones that are tightly bound—to relatively massive companions—are likely to remain in their binary orbit. Hence accretion clouds become an important factor. Most binary pulsars are unlikely to appear in radio surveys as their accretion clouds and the coronas of their companions would generally have plasma frequencies in excess of the radio. (The only binary pulsar found by Hulse and Taylor had a compact companion; one might expect many more with main sequence companions) If they are blanketed by more than 1 g cm^{-2} , their pulsation could not be detected at any frequency, and if by more than $\sim 30 \text{ g cm}^{-2}$, no non-thermal photons are likely to escape because they would first be degraded to energies below 10 keV, where they would then be absorbed by partially ionised iron and oxygen^{13,17}.

Cyg X3, generally presumed to be a pulsar in a binary system, is blanketed by $\sim 10 \text{ g cm}^{-2}$ (ref. 10), and is deduced to be a pulsar only by virtue of its γ -ray emission. Cyg X3 itself may be detectable in high energy neutrinos if it emits $\geq 10^{35}$ erg s⁻¹ in high energy protons. There may also be a whole class of objects like Cyg X3, but obscured by much thicker envelopes.

Hefand and Tadamaru¹⁶ noted a distinct class of pulsars with very low transverse velocity. To account for this, they suggest

that $\sim 20\%$ of all pulsars form in close binary systems, probably in a 'cataclysmic binary' sequence such as outlined here. (The pulsar is later released gently from the binary as the companion sheds mass.) High energy neutrino astronomy would provide an opportunity to test such a hypothesis. Conversely, their hypothesis suggests that there may be many interesting astrophysical phenomena to observe with neutrino telescopes. We emphasise that if high energy particles generated by the pulsar manifest themselves by bombarding the corona or envelope of the companion star, the mesons and photons they produce are directed into the star. Hence high energy neutrino astronomy may be the only way to detect high energy phenomena in such systems.

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Constraints on the corona model for Sirius B

SINCE the discovery of Sirius as a soft X-ray source¹, two models have been proposed to account for the emission—thermal emission from deep layers of an almost pure hydrogen atmosphere², and an acoustically heated corona around the white dwarf (WD) Sirius B (ref. 3). Both models seem unsatisfactory in some respects: the thermal emission model does not agree with the recently revised upper limits found from extreme UV observations⁴, and the T_{eff} of Sirius B is much too large for H-convection. In this note we re-examine the constraints on the corona model in the light of historical records suggesting a possible pseudo-red giant phase a few thousand years ago.

Sirius B does have an H-atmosphere, and with T_{eff} being $32,000 \pm 1,000 \text{ K}$ (ref. 5), the atmosphere is stable against convection. If it is accepted that the corona is due to the dissipation of the acoustic flux produced in subatmospheric convective layers, then for Sirius B to have a corona there must be an He-convective region beneath the atmospheric H-layers. Fontaine⁶ argues that in this case the acoustic flux produced would be much too low to satisfy the energy requirement to heat the corona. However, the theory of corona formation is not well developed⁷, and the computation of acoustic fluxes is critically dependent on the theory of superadiabatic convection. Hence, if convection survives in the subatmosphere of Sirius B, we cannot exclude the corona model. In fact, Lampton and Mewe⁸ recently revised the energetic requirements for white dwarf coronae and

claim that the corona around Sirius B may exist, provided that the H-layer is very thin.

The theory of white dwarf envelopes is already sufficiently good to quantify what 'very thin' means. The H mass M_H must be larger than the mass which provides Rosseland optical depth $\tau = 1$, as we see an H-atmosphere, and that it accreted H from the stellar wind of Sirius A during the white dwarf lifetime ($t = 3\text{--}4 \times 10^7$ yr, ref. 12). Since it is now clear^{13,14} that He white dwarfs do not accrete from interstellar matter at the rates predicted by Bondi's accretion formula¹⁵, but the geometrical accretion rate is appropriate¹⁰, one may derive the mass loss rate ($\dot{M}$) from Sirius A which accounts for the required M_H , simply as:

$$M_H = \dot{M} \pi R_s^2 / (4 \pi d^2)$$

where R_s is the Sirius B radius (5.5×10^8 cm) and d the system separation (20 AU). This relation provides $3 \times 10^{-13} \leq \dot{M} < 2 \times 10^{-12} M_\odot \text{ yr}^{-1}$, which is not an unreasonable rate for a $2M_\odot$ star. Therefore, the corona model for Sirius B cannot be excluded on the basis of these arguments. If the white dwarf, corona-like emission were confirmed, we should know, with an uncertainty of a factor of only 10, the rate of mass loss from Sirius A.

However, we feel that the historical record of Sirius being a red star should not be dismissed as unpalatable. Most of the references to this (Greek, Roman, Egyptian) were collected at the end of last century by Schiaparelli^{16,17}, who did not believe that Sirius could have changed colour, because, in his time, the theory of stellar evolution predicted that stars evolved from white to red, and then to total darkness—'as a piece of iron cooling from white heat'. Further historical quotations—not so complete—are given by See¹⁸, who, unlike Schiaparelli, believed that the historical evidence strongly suggested that a colour change had taken place. If the historical records are to be believed, Sirius must have changed colour from red to white between the epoch of Ptolemy (AD 100) and the epoch of Al-Sûfi (AD 980), within 800 yr.

We now know with reasonable certainty that the age of Sirius as a white dwarf cannot be so low, but we also know that hot white dwarfs are not always 'normal' stars: instabilities connected to H burning in thin shells can be found, and these may last few thousand years¹⁹. Therefore, we cannot rule out the possibility that Sirius B went through a pseudo-red giant phase a few thousand years ago.

In this context, we emphasise that the sudden expansion of the outer envelope of a white dwarf is always related to H burning, which may occur only if the H envelope of the white dwarf is large ($\geq 10^{28}$ g), and the expansion for a few thousand years is never able to completely remove this envelope. In other words, if the historical record is true, then the H envelope of Sirius B is orders of magnitude larger than the envelope required for He convection to survive, and the corona model is wrong.

Hence, if it is found that Sirius B does indeed produce a corona-like emission, and if further study of historical records and archaeological evidence indicates a pseudo-red giant phase, the theory of generation of a corona by acoustic flux dissipation must be seriously questioned. The acoustic flux model will also be in doubt, if the coronal X-emission is shown to arise from Sirius A, since Sirius A, like Sirius B, cannot have subatmospheric convection.

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If the Sun has a companion . . .

HARRISON¹ has suggested on the basis of pulsar data that the Sun might have a companion. This letter discusses some of the implications if such a companion is a compact object, more precisely a neutron star (NS) or a black hole (BH). Using existing accretion results, it is argued that it is impossible to rule out either a NS or a BH as a possible candidate. However, constraints can be placed on the allowed mass range and/or radiation mechanisms. If such a compact object exists, the probability of observing the gravitational lens effect can be substantial.

The case for a low-mass star, a black or a white dwarf has been discussed by Henricks and Staller² who concluded that such an object would be conspicuous in the visible or IR sky and would probably have already been detected. The situation for a NS or a BH is not quite so clear and one needs to consider in more detail the luminosity achieved through accretion.

Because a weakly bound binary system may not survive the explosion leading to a NS or a BH, we only consider the simple case of a close encounter between our Sun and its postulated temporary companion. The probability of such an encounter or its perturbing effects on planetary motions will not be discussed here. The accretion rate $\dot{M}$ is of the order³ $\dot{M} \approx 4\pi G^2 M^2 \rho_0 / (a_0^2 + v^2)^{3/2}$ where ρ_0 and a_0 are the density of the unperturbed ambient gas and the corresponding speed of sound, and M and v are the mass and velocity of the compact object. As $a_0 \approx 10 \text{ km s}^{-1} (T/10^4 \text{ K})^{1/2}$ and as we expect $v \geq 10 \text{ km s}^{-1}$, the accretion rate⁴ will be of the order $\dot{M} \approx 3 \times 10^{-16} (M/M_\odot)^2 (n_0/1 \text{ cm}^{-3}) (v/10 \text{ km s}^{-1})^{-3} M_\odot \text{ yr}^{-1}$, where n_0 is the particle number density of the unperturbed gas. Taking first the case of a BH and using previous results obtained for spherical accretion from a medium at $T \approx 10^4 \text{ K}$ (as a_0 is then of the order 10 km s^{-1} , this is equivalent to considering a BH moving through the interstellar medium at $v \approx 10 \text{ km s}^{-1}$), we write the total luminosity in the form⁴⁻⁶

$$L_{\text{BH}} = \Lambda_{\text{BH}} (M/M_\odot)^3 (n_0/1 \text{ cm}^{-3})^2 (v/10 \text{ km s}^{-1})^{-3}$$

where the quantity Λ_{BH} and the spectral energy distribution depend on the details of the model. (For the models considered here, $\Lambda_{\text{BH}} \approx 10^{21}\text{--}10^{29} \text{ erg s}^{-1}$.) As for accretion on to a NS, most (if not all) of the energy is radiated away so that $L_{\text{NS}} =$

$\eta GMR^{-1} \dot{M}$, where R is the radius of the NS. Taking $\eta \approx 0.3$ and $M/R \sim 10^5 M_\odot/R_\odot$ for a $1 M_\odot$ NS, and assuming a crude mass-radius relationship of the form $MR^3 = \text{constant}$, the total luminosity takes the form $L_{\text{NS}} = \Lambda_{\text{NS}} (M/M_\odot)^{10/3} (n_0/1 \text{ cm}^{-3})(v/10 \text{ km s}^{-1})^{-3}$, where $\Lambda_{\text{NS}} \approx 10^{30} \text{ erg s}^{-1}$. Again, the spectrum depends on the detailed processes by which the radiation is emitted.

Taking the distance d to the companion (as determined by the required acceleration of order $10^{-6} \text{ cm s}^{-2}$ for the Solar System) to be $d = 800(M/M_\odot)^{1/2} \text{ AU}$, we can now obtain the apparent visual magnitude V and expected X-ray intensity I in c.p.s. (counts per second) in the range 2–6 keV (1 count $= 1.7 \times 10^{-11} \text{ erg cm}^{-2}$), provided the spectral distribution of the radiation is known. For different parameters the quantities V and I vary according to

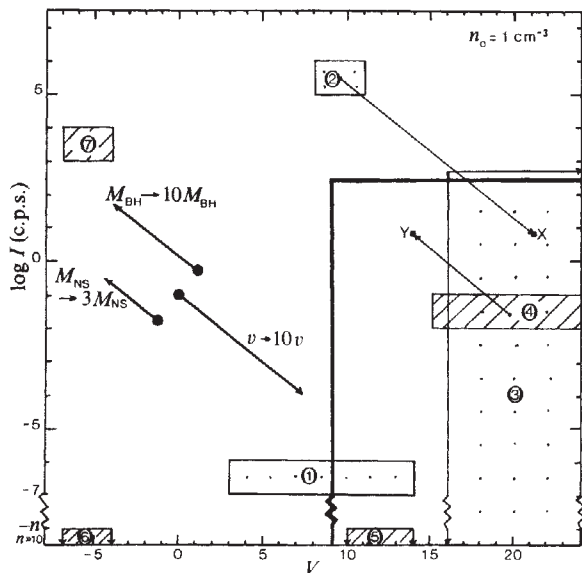
$$I = \text{const} \times M^{a-1} n_0^b v^{-3}$$

$$V = \text{const} + 2.5((1-a) \log M - b \log n_0 + 3 \log v) \quad (1)$$

with $(a, b) = (3, 2)$ for a BH and $(10/3, 1)$ for a NS. With respect to the X-ray intensity I , we note that on the basis of X-ray data⁸ there are 14 sources in the expected¹ direction of the companion, that is $17 \text{ h } 30 \text{ min} < \text{RA} < 19 \text{ h } 52 \text{ min}$, $-27^\circ < \delta < 32^\circ$. Six of these are close to the general direction to the galactic centre and thus probably associated with it, so we need not consider them. Of the eight sources left, all have an intensity $< 270 \text{ c.p.s.}$

Figure 1 shows V plotted against $\log I$ giving the results corresponding to various radiation processes around accreting NS or BH for the case $n_0 = 1 \text{ cm}^{-3}$, $v = 10 \text{ km s}^{-1}$ and $M_{\text{NS}} = 1 M_\odot$, $M_{\text{BH}} = 10 M_\odot$. The fact that each case is represented by an area instead of a point reflects the uncertainties in estimating the amount of energy emitted in the visible and X-ray bands. (The IR magnitude K also generally correlates with the V magnitude so that a bright visible object would also be a bright IR object.) The three NS results are labelled 1, 2 and 3 on Fig. 1.

Fig. 1 V magnitude and (2–6 keV) X-ray intensity I corresponding to various radiation processes, discussed in the text, around accreting NS (dotted areas 1, 2 and 3) and BH (hatched areas 4, 5, 6 and 7). The parameters used are: $n_0 = 1 \text{ cm}^{-3}$, $M_{\text{NS}} = 1 M_\odot$, $M_{\text{BH}} = 10 M_\odot$ and $v = 10 \text{ km s}^{-1}$. The three arrows on the left of the diagram illustrate the shift in the position of three arbitrary points (●) if M and v are changed as shown. Arrows leading to points marked X and Y refer to specific changes discussed in the text. An object could have possibly remained undetected until now only if its corresponding point on the diagram lies inside the region indicated by the heavy line, that is $V \geq 9$, $I \leq 270 \text{ c.p.s.}$



As the emitted spectrum is complex and depends strongly on the actual physical processes (for a review of accretion on to a NS, see ref. 7), we have not tried to represent realistic situations. Instead, we have assumed some simple radiation mechanisms to illustrate the wide range of possible results. Hence, cases 1 and 2 correspond respectively to black-body emission at $T \approx 10^5$ – 10^6 K and bremsstrahlung at $T \approx 10^9 \text{ K}$ (in the latter case, comptonisation if taken into account would augment the high-energy end of the spectrum). In case 3, a shock above the surface is assumed to heat the electrons to a high temperature of the order of 10^{12} K , and V and I are estimated by assuming that the bulk of the energy is in the form of γ rays and that the spectral distribution does not increase at lower energies. The four BH results, labelled 4, 5, 6 and 7, were obtained from earlier work on accretion. For the chosen models, the emission is predominantly due, in case 4 to radiative recombination and bremsstrahlung⁴, in cases 5 and 6 to electron synchrotron radiation^{5,6,14}, and in case 7 to bremsstrahlung, thermal and cosmic ray electron synchrotron radiation¹⁵.

Clearly, for either a NS or a BH, the results are very sensitive to the details of the emission process. In particular, a NS companion can certainly not be ruled out, especially because increasing the velocity to 100 km s^{-1} , and taking a lower mass (all observed NS masses⁹ seem to lie in the range $0.3 \leq M/M_\odot \leq 1.2$) substantially favour the possible existence of a NS companion (all points get shifted diagonally downwards parallel to the arrows on the diagram). As for a BH candidate, if Shvartzman's model or Mészáros' is the correct one (areas 6 or 7 on Fig. 1, large synchrotron luminosity including contribution from cosmic ray electrons in Mészáros' case), then a BH companion, in order not to be one of the brightest objects in the visible sky, would have to have either $v \gg 10 \text{ km s}^{-1}$ or $M \ll 10 M_\odot$ or both (for example, a $1 M_\odot$ BH moving with velocity $v = 100 \text{ km s}^{-1}$ would have $V \approx 9$). Note that a BH of $1 M_\odot$ would have to be primordial in origin. It is interesting that, taking the maximum allowed cosmological density¹⁰ of BH and a velocity of the order 100 km s^{-1} , an encounter at the required distance every 10^8 – 10^9 yr is expected independent of the mass of the BH (both the number of BH, and the square of the distance depend linearly on the mass of the BH). On the other hand, taking Shapiro's model or equivalently assuming the magnetic field to be relatively unimportant (areas 4 and 5 on Fig. 1), then one sees that a BH with $M \leq 10 M_\odot$ and $v \geq 10 \text{ km s}^{-1}$ could easily have remained undetected until now. Wright¹¹ has suggested a supermassive black hole of $M > 6 \times 10^3 M_\odot$ moving with $v > 9 \text{ km s}^{-1}$ as a possible solar companion. Compared to a $10 M_\odot$ BH, such a BH would have a luminosity larger by a factor of order 4×10^5 (corresponding to $\sim 14 \text{ mag}$). Regardless of the accretion model adopted, this BH would be very conspicuous in both or either of the visible and X-ray sky and is thus highly unlikely as a candidate.

Could one of the already observed X-ray sources be this postulated companion? Of the eight aforementioned X-ray sources listed in the 3U catalogue⁸, one, 3U1832–23, lies on the ecliptic (thus minimally disturbing the orbit of the planets). For this source $I = 6.9 \pm 0.9 \text{ c.p.s.}$ If this source were the solar companion, then we could certainly rule out cases 1, 5, 6 and 7. On the other hand, assuming the kind of simple spectra used in Fig. 1, a NS having $M \approx 1 M_\odot$, $v \approx 10 \text{ km s}^{-1}$ could possibly be a candidate but only if electrons were heated to a very high temperature (case 3, but then it should also be a strong γ -ray emitter). Another possibility would be a NS with a basically thermal bremsstrahlung spectrum at $T \approx 10^9 \text{ K}$ (case 2) provided $(M/M_\odot)^{7/3}(v/10 \text{ km s}^{-1})^{-3} \approx 2 \times 10^{-5}$ (this change of parameters would shift the position of a point in dotted area 2 diagonally to a point X at $V \approx 21$), a typical example being $M \approx 0.3 M_\odot$, $v \approx 140 \text{ km s}^{-1}$. An accreting BH with a simple bremsstrahlung spectrum (case 4) could also be a candidate if $(M/M_\odot)^2(v/10 \text{ km s}^{-1})^{-3} \approx 200$. This resulting increase in luminosity (shift to a point Y on Fig. 1) could be achieved, for example, with a $150 M_\odot$ BH moving at $v = 10 \text{ km s}^{-1}$. Such a BH would be at $d \approx 9,000 \text{ AU}$ and would have a proper motion

$\mu_0 \approx 45 \alpha \text{ arc s yr}^{-1}$ and parallax $\pi \approx 20 \text{ arc s}$, with α the ratio of tangential to total velocity.

It has been assumed so far that a value of $n_0 = 1 \text{ cm}^{-3}$ is typical of the interstellar medium. The actual density outside the Solar System¹⁶ may well, however, be only of the order 0.1 cm^{-3} . If this is the case, the luminosities derived from accretion are then reduced by a factor of 100 for a BH and 10 for a NS, thus making any such compact object even less conspicuous.

Another aspect of interest is the question of whether gravitational lensing by the postulated companion can have observable consequences. Wright¹¹ has briefly commented on this point. We calculate here the probability that the compact object will act as a moving lens causing an angular displacement and brightening up of distant background stars. To appreciate the angles involved, a typical angle is the angular radius of the annular image of a distant source when there is perfect alignment. This angle γ_0 (half the angle α_0 used by Refsdal¹²) is given by

$$\gamma_0 = 1.47 \text{ arc s } (M/M_\odot)^{1/4}$$

where the distance-mass relation is used for the postulated companion and the fact that this distance is considerably smaller than the star-deflector distance. For a seeing disk of 1 arc s, the above relationship shows that the deflector and primary image (the only one we consider here) are separately resolved. The amplification factor, defined as the ratio of the lensed flux to the unlensed flux is given by¹² $A = \frac{1}{4}(2 + (\alpha/\beta) + (\beta/\alpha))$, where $\alpha^2 = \alpha_0^2 + \beta^2$, $\alpha_0 = 2\gamma_0$, and β is the angular separation between the position of the deflector and that of the source in the absence of light deflection ($\beta = 0$ for perfect alignment). The corresponding magnitude change is $\Delta m = 2.5 \log A$. The angular position of the primary image with respect to the deflector is given by $\alpha_* = \frac{1}{2}(\alpha + \beta)$ so that the position of the image is apparently shifted by the angle $\delta = \alpha_* - \beta = (\alpha - \beta)/2$. Defining a characteristic transit time T as the time during which the magnitude of the image m_* satisfies $m - \Delta m/2 \leq m_* \leq m + \Delta m/2$, m being the unlensed magnitude, we find $T = 2(\beta_{1/2}^2 - \beta^2)^{1/2} \mu^{-1} \text{ yr}$ where $\beta_{1/2}$ is the value of β corresponding to a change $\Delta m/2$ and μ is the net angular velocity of the deflecting mass, including proper motion μ_0 and parallax π (in arc s yr⁻¹ if β is in arc s).

Given a plate limit m_{lim} , the probability P of observing an angular displacement of magnitude $\geq \delta$ or equivalently a change in magnitude $\geq \Delta m$ can be computed by summing the separate contributions P_m corresponding to background stars of magnitude m , $P_m = 2\beta\mu A_m(60)^{-4} \text{ yr}^{-1}$, where A_m is the number of stars¹³ per square degree in the visual magnitude range $m \pm \frac{1}{2}$ and β and μ are expressed in arc s and arc s yr⁻¹, respectively (the summation $P = \sum P_m$ formally extends to ∞ ; however, for stars with $m > m_{\text{lim}}$, the required change in magnitude is not Δm but $\Delta m + (m - m_{\text{lim}})$, thus giving an observable change of Δm).

Keeping all other parameters constant (such as the magnitude and direction of the tangential velocity), the various quantities scale with the mass as follows: $\pi = c_1 M^{-1/2}$, $\mu_0 = c_2 M^{-1/2}$ and hence $\mu = c_3 M^{-1/2}$, $P = c_4 M^{-1/4}$ and $T = c_5 M^{3/4}$, where the c 's are all constant. The quantity $N = PT$ where $\bar{T}$ is an average transit time (stars with $m > m_{\text{lim}}$ have a shorter transit time with a lower probability of occurrence) is entirely independent of μ and scales as $M^{1/2}$. It is a measure of the number of days in a year where gravitational lensing is in progress. (We can interpret a value of N larger than 365 as meaning that a number of order $N/365$ lensings are occurring simultaneously.)

The results of the calculations are shown in Table 1 and Fig. 2 for a $1 M_\odot$ object. The chosen net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$ has the same value as the proper motion of a $1 M_\odot$ object having a tangential velocity of 10 km s^{-1} . (Its annual parallax would be 250 arc s .) These results are easily generalised to different values of the parameters by using the appropriate scaling laws.

Table 1 shows that the angular shift is a much more noticeable effect than the accompanying brightening up. For a $1 M_\odot$ object, it is also clear from Fig. 1 that, unless $m_{\text{lim}} \geq 21$, one does not expect numerous lensings giving a deflection of more than 1 arc s

Table 1 Typical parameters characterising the gravitational lens effect of a nearby $1 M_\odot$ object having net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$

Δm	0.0002	0.001	0.01	0.05	0.1	0.5	1.0	2.0
α_* (arc s)	12.6	8.44	4.75	3.19	2.70	1.89	1.67	1.53
β (arc s)	12.4	8.18	4.30	2.51	1.90	0.74	0.37	1.27
δ (arc s)	0.17	0.26	0.46	0.68	0.80	1.15	1.29	1.41
T (d)	10.40	6.96	3.90	2.56	2.12	1.20	0.82	0.46

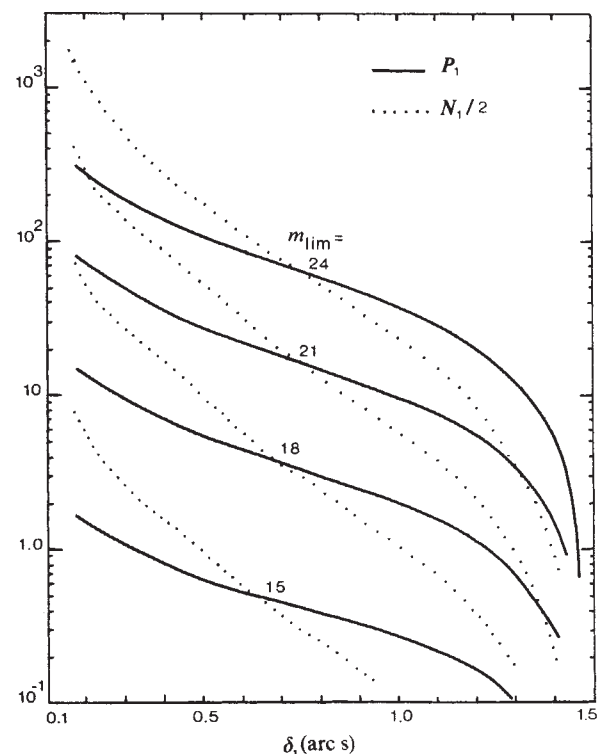
See text for scaling laws.

or $\Delta m \approx 0.1$. However, the probability of observing a deflection of order 0.4 arc s (not an unreasonable value) rises to 0.8, 7, 35 and 140 per yr for limiting plate magnitudes of respectively 15, 18, 21 and 24, each transit lasting about 4 d. Hence, a $1 M_\odot$ object would be detected by (say) taking four or more plates down to $m_{\text{lim}} = 21$ at intervals of three nights.

As for a $150 M_\odot$ BH (hypothetical 3U1832-23 case), a deflection $\delta \geq 0.5 \text{ arc s}$ (corresponding to $\delta_1 \approx 0.14 \text{ arc s}$ on Fig. 2) should be observable roughly 0.6, 6, 30 and 120 times per yr for $m_{\text{lim}} = 15, 18, 21$ and 24, respectively, the transit time now being of order 515 d (about 1.4 yr). Note that the probability (transit time) increases (decreases) by a factor of 10 if we allow a tangential velocity of the order 100 km s^{-1} . On the other hand, a NS or primordial BH of $0.3 M_\odot$ will produce a deflection of the order 0.4 arc s a number of 0.8, 8, 35 and 135 times per yr with a transit time of only 1.4 d.

It should be pointed out here that all calculations are based on mean (with respect to galactic latitude) values for A_m , thus underestimating its value on the galactic plane and hence the probabilities by a factor of the order 3-4. And, of course, if the presumed compact object is brighter than the limiting magnitude, its proper motion alone will make it conspicuous.

Fig. 2 The quantity $N_1/2$ and probability P_1 of observing a gravitational deflection greater than or of the order δ_1 for a $1 M_\odot$ object (hence the subscript) having net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$. The corresponding quantities for other masses, other parameters being the same are given by $P = P_1(M/M_\odot)^{-1/4}$, $N = N_1(M/M_\odot)^{1/2}$ and $\delta = \delta_1(M/M_\odot)^{1/4}$ (strictly speaking P_1 is the expected number of lensings per year).



In conclusion, a temporary companion to our Sun in the form of a NS or a BH could have remained undetected until now. Constraints can be placed, however, on the allowed type of spectrum and/or mass and velocity ranges accessible. In particular, if $n_0 = 1 \text{ cm}^{-3}$ and if the magnetic field plays an important part, we can almost certainly rule out any BH which has $M > 1 M_\odot$ and $v < 100 \text{ km s}^{-1}$. On the other hand, if an encounter with a compact NS or BH is in progress, it could be possible to observe the gravitational lens effect, especially if one knew more precisely in which direction to look. Perhaps some photographic observations of the error boxes associated with the eight aforementioned Uhuru sources would be worthwhile, starting with 3U1832-23. At worst the data obtained could be useful for alternative work such as variable star or proper motion survey; at best, one will have the satisfaction of having observed the (most likely) closest compact object.

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Evidence for long-period global oscillations of the Sun

THE results of our observations of the solar potassium resonance absorption line at $7,699 \text{ \AA}$ using integrated solar light from the McMath telescope at Kitt Peak together with an atomic beam resonance scattering apparatus are reported here. In two long runs on successive days we observed wavelength oscillations of large amplitude with a period close to 160 min. If, as we believe, these oscillations are produced by motions of the photosphere, they provide evidence for oscillations of the entire Sun. We describe our experimental method, the analysis of the observations, and the results we obtain, and compare these with previous results.

Resonance scattering of light from a beam of free atoms is an ideal technique for making precise absolute measurements of the shift in wavelength of the light relative to the reference wavelength of the beam atoms. We have used this technique previously to measure the solar gravitational redshift¹ and to observe the 5-min radial oscillation of small regions of the photosphere². We give here only a brief outline of the method as modified for our present observations.

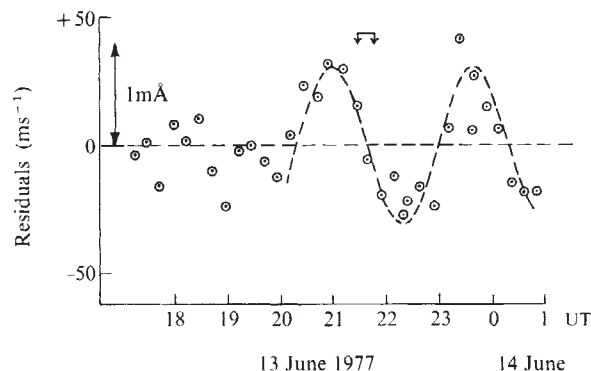
Flat mirrors placed in the main beam of the McMath telescope directed integrated sunlight vertically downwards into our apparatus, where it passed through a $3/16''$ entrance aperture, a $7,699\text{-\AA}$ interference filter with a $16\text{-\AA}$ FWHM and a motor-driven circular polariser unit which alternated the sense of circular polarisation at a frequency of about 23 Hz. The light then passed through a lens and the upper pole piece of a variable-field permanent magnet into a vacuum chamber placed

between the magnet poles where it was focused on a horizontal beam of potassium atoms produced in a nearby oven. Unscattered light was absorbed in a blackened tube placed below the beam while light resonantly scattered in a direction perpendicular to the beam passed through a short horizontal fibre optics light pipe to a dry-ice-cooled photomultiplier tube. The magnetic field was set at its maximum value, roughly 1,800 G, to produce a Zeeman splitting of the beam atom resonance absorption line into two lines whose wavelengths corresponded to the steeply sloping sides of the integrated solar potassium line. The two opposite senses of circular polarisation of the input light produced resonance scattering by beam atoms at these two different wavelengths, so that the system switched back and forth between opposite sides of the solar line at a frequency of 23 Hz. For small wavelength shifts of the solar line, the amplitude of the photomultiplier output signal at 23 Hz was proportional to the shift. This signal was fed to a Keithley 417 picoammeter whose output went to a Keithley 822 phase-sensitive detector (PSD) provided with a 23-Hz reference signal from the circular polariser unit. The d.c. output of the PSD was measured by a Keithley 172 digital multimeter and recorded by a Keithley 750 printer.

We incorporated several features designed to eliminate possible slow variations in the telescope alignment and instrumental effects. First, we diverted light from around the entrance aperture to an image-forming system so arranged that when the image was kept accurately fixed in position by photoelectric guiders the direction of the integrated light entering the aperture was also kept fixed. Second, we continuously monitored on a chart recorder the average value of the picoammeter output signal corresponding to the average of the scattered light intensity from both sides of the solar line as well as the stray light scattered from the walls of the vacuum chamber. At intervals we recorded this signal with the potassium beam turned off, to measure the stray light alone. Third, at intervals we recorded the PSD output with the beam turned off, to measure instrumental effects such as polariser asymmetry.

Our observations were made on 13 and 14 June 1977; we report here only those made during the same continuous time interval on both days. The PSD time constant was 10 s and in our data analysis we used recorded PSD outputs at 1.85-min intervals. After subtracting from each PSD output value the value with atomic beam off and then normalising it using the net beam-on minus beam-off average picoammeter output, we carried out a least-squares fit of the resulting data to the known variation of wavelength with time produced by the Earth's motion. This known variation was used to calibrate our apparatus. Finally, we averaged the residuals in groups of eight, corresponding to a time interval of 14.8 min, and plotted them in Figs 1 and 2.

Fig. 1 Residual wavelength variations of the solar potassium absorption line at $7,699 \text{ \AA}$ using integrated light. Each point is the average of eight residuals over a 14.8-min period. The dashed sine curve has a period of 160 min and its first peak occurs at 20.58 UT. The expected location of a peak, based on the data of Kotov *et al.* lies in the range indicated by the two arrows. A positive residual corresponds to ascending motion on the Sun.



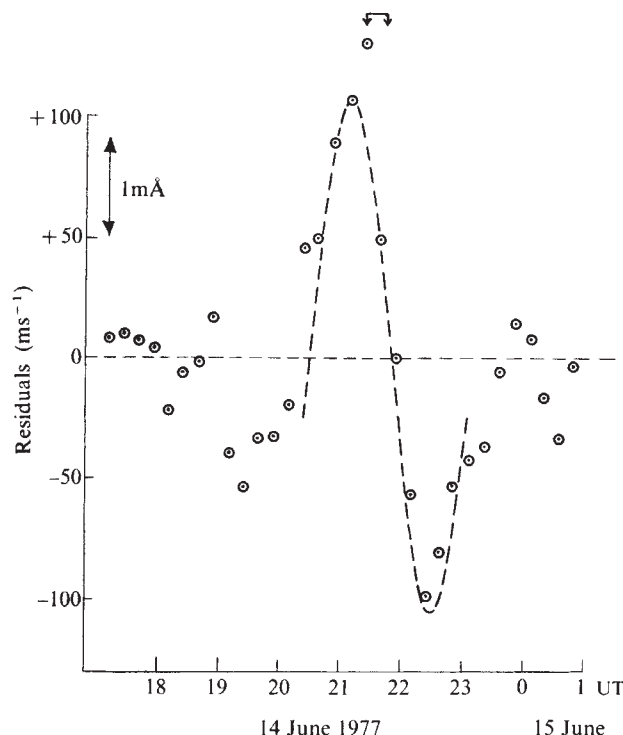


Fig. 2 The dashed sine curve has its peak at 21.10 UT. See legend to Fig. 1 for further details.

At the start of both runs the data seem to scatter randomly. Regular variations then appear with considerably greater amplitude than the noise. Kotov *et al.*³ have presented evidence for periodic variations in the wavelength of an iron line at 5,124 Å, with several different periods, one of which is 160.004 min and has an associated amplitude of about 1 m s⁻¹. These variations remained in phase over a three-year period from 1974 to 1976 and were consistent with the more limited observations of Brookes *et al.*⁴ on the potassium line with a technique similar to ours. Figures 1 and 2 show 160-min sine curves fitted by eye to our data: 160 min is apparently very closely the proper period, but the locations of the peaks only roughly agree with the expected locations based on our extrapolation of the data of Kotov *et al.* which lie in the ranges indicated on the figures by double arrows. (To make this extrapolation we assumed that a peak occurred between 4 h 47.5 min and 5 h 7.5 min UT on 4 August 1974, and that the oscillation period was 160.004 min. See Fig. 6 of ref. 3. If the period is actually 160 min then nine complete oscillations will occupy exactly 24 h so that on successive days the peaks should occur at the same times. We observed a peak on 14 June at 21.10 UT, which is 12 min later than the peak at 20.58 UT on 13 June. A possible explanation is that the period of oscillation of the potassium line is 161.3 min. This would link our data on the two successive days very nicely as well as fitting the data even better.

The power spectra calculated for these two runs showed enhanced power in the range of long periods and also other peaks but we do not trust the results because of the limited amount of data available. It seems that when there are large oscillations obviously present in a data string, such an analysis is not very useful and is difficult to justify, particularly when each run is only three oscillation periods long. Furthermore, we are wary of attaching too great a significance to the specific periods associated with peaks in the power spectrum as the periods for which power is calculated are determined by the total data string length and are widely separated.

We believe that our method of data analysis has eliminated possible instrumental variations as the cause of the observed wavelength oscillations. We do not see how variations in the Earth's atmosphere could cause such an effect and we conclude

that we have observed a solar effect associated with global oscillation. The large amplitude of the oscillation relative to that observed by Kotov *et al.* as well as the apparent difference in phase still require explanation. Detailed discussion of the expected oscillation periods according to various solar models can be found in ref. 3 and other references listed there.

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Measurement of ¹⁰Be in 1,000- and 5,000-year-old Antarctic ice

MEASUREMENT of radionuclides by nuclear accelerator spectrometry may lead to wider application of measurement of long-lived cosmogenic isotopes to archeological, geophysical and astrophysical problems (see ref. 1 for further details of the technique). We previously² discussed the prospects for the isotope ¹⁰Be and reported the successful measurement of this isotope using both the internal² and the external³ ion sources of the Grenoble cyclotron. ¹⁰Be (half life = 1.5 × 10⁶ yr; ref. 4) is formed by the interaction of cosmic rays with the Earth's atmosphere, and then precipitated out into various geophysical reservoirs such as marine sediments and polar ice⁵. The ¹⁰Be profile in these reservoirs can thus be used to search for evidence of historical variations in parameters (geomagnetic field, solar modulation, primary cosmic ray intensity) which influence the deposition rate. We report here the use of the accelerator technique for the determination of ¹⁰Be concentrations in samples of Antarctic ice believed to be about 1,000 and 5,000 yr old. The only published measurement for ¹⁰Be in polar deposits is that of McCorkell *et al.*⁶ who made their measurement on a sample extracted from 1.2 × 10⁶ l of Greenland ice. The present measurements were made on samples from 10 l. We believe this difference is indicative of the tremendous potential that is offered by the accelerator technique.

The samples studied were taken by a group from the Laboratoire de Glaciologie de Grenoble during the recent successful recovery of a 900-m ice core at Dome C in East Antarctica⁷. During the coring operation, a certain amount of meltwater occurs. This water was recovered and passed through cation exchange resin in the form of resin impregnated filters (Acropor SA-6404, Gelman). The resins were changed at regular intervals. Two of the cation disks were used for the present investigation. The depth and quantity of water to which they were exposed are given in Table 1.

Be was extracted from the resins by soaking them in 3M HCl for several hours (a test with ⁷Be confirmed that 100% extraction was obtained in these conditions). A standardised carrier solution, containing ~4 mg of ⁹Be was then added to the HCl. The mixture was neutralised and the precipitate of Be(OH)₂ converted to BeO by heating. Pellets of sintered BeO were then fabricated by the procedure described in ref. 2 for introduction into the cyclotron ion source.

The experimental procedure was basically that described in ref. 3 using the external ion source of the cyclotron. Several of the improvements discussed in ref. 3 have been implemented,

Table 1 ^{10}Be determinations and sample details

Sample	Depth (m)	Estimated age* (yr BP)	Vol. of water (l)	$^{10}\text{Be}/^9\text{Be}^\dagger$ ($\times 10^{-13}$)	^{10}Be ($\times 10^4$ atoms per g ice)
RD-14	70.9–75.7	1,000	11.2	7.6 ± 2.5	2.00 ± 0.67
RD-18	235–242	5,000	10.2	11.2 ± 2.8	3.22 ± 0.81
Standard‡	—	—	—	8.1 ± 1.9	—

* Ages are preliminary estimates based on model calculations (C. Lorius, personal communication).

† These ratios are normalised to known standard having $^{10}\text{Be}/^9\text{Be} = 8.24 \times 10^{-12}$.

‡ This standard has a known ratio of 8.30×10^{-13} .

including replacement of the single Si detector by a $\Delta E(8\mu\text{m})$ -E telescope, a 'live-time' clock to take into account high voltage breakdowns, and rapid switching between ^9Be and ^{10}Be at the source by means of changing the extraction voltage rather than the magnetic field. In addition all beam lines have been tuned initially using $^{10}\text{B}^{2+}$ rather than $^{20}\text{Ne}^{4+}$ and this has resulted in an improved transmission efficiency. The most important change was use of a significantly more intense ($\sim 3\mu\text{A}$) output from the ion source. A more detailed description of these modifications will be given elsewhere. For the present discussion the most important effect was that we were able to improve our sensitivity by about two orders of magnitude compared to ref. 3 and so measure samples with $^{10}\text{Be}/^9\text{Be}$ ratios as small as 10^{-12} .

The analysis of the ice samples was made by comparing their $^{10}\text{Be}/^9\text{Be}$ ratio to those of standardised $^{10}\text{Be}/^9\text{Be}$ samples prepared by the method outlined in ref. 2. To avoid contamination problems measurements were made on a 'clean' ion source in the following order; standard ($^{10}\text{Be}/^9\text{Be} = 8.30 \times 10^{-13}$), ice samples, standard ($^{10}\text{Be}/^9\text{Be} = 8.24 \times 10^{-12}$). Each measurement included 20 min of ^{10}Be counting, with the ^9Be current being recorded every 5 min. A blank run gave no counts in 10 min which is equivalent to a background of less than 10% of the ice samples.

Knowing the $^{10}\text{Be}/^9\text{Be}$ ratio and the quantity of ^9Be added initially, we obtain directly the number of ^{10}Be atoms in the original sample. It is interesting to note that the chemical yield of our processing treatment does not enter into such a calculation since we are at all times working with isotope ratios. Also, as our standards have been determined by a mass spectrometric measurement², even the uncertainty associated with the ^{10}Be half life does not intervene. On the other hand, one assumption we have been forced to make is that all the ^{10}Be initially in the meltwater has been recovered in the resin. Because of the well known tendency of carrier-free Be to hydrolyse in neutral solution and adsorb on container surfaces, this assumption needs to be verified. We hope to be able to do this in the future by measuring the ^{10}Be taken directly from an equivalent quantity of ice in the same core.

The results of our measurements are summarised in Table 1. As the ^{10}Be count rates for the ice samples were 1 to 2 c.p.m., the uncertainties are dominated by statistics. We point out, however, that this limitation in the present circumstances was due to the lack of accelerator time and not to running out of sample. In fact by weighing our samples before and after we were able to estimate that they were consumed at the rate of $\sim 0.05\text{ g min}^{-1}$.

The present experiment is a prelude to a more general project for measuring complete ^{10}Be 'profiles' in polar ice and marine sediment cores, in order to investigate possible ^{10}Be variations with time and their eventual implications. In fact, the two ice samples in Table 1 do have a concentration difference (which is, however, not statistically very significant). It is tempting to attribute this to the difference in geomagnetic field at the two epochs. It is generally believed that the intensity of this field 5,000 yr ago was only about half of that 1,000 yr ago⁸, and thus ^{10}Be production would be expected to be enhanced at the earlier period. Indeed, this was the reason for choosing the samples

from these periods. However, in view of the limited precision of the present measurements and the fact that we are dealing with only two samples, we content ourselves for the moment with simply taking the mean of the two results as representing a long-term average for the ^{10}Be deposition rate at the site tested.

Using an estimated annual precipitation rate of 3.7 cm of water at the site of our samples (R. Delmas, personal communication), our average ^{10}Be concentration (2.6×10^4 atoms per g ice) represents a ^{10}Be deposition rate of 3.1×10^{-3} atoms $\text{cm}^{-2} \text{s}^{-1}$. This is much less than the value $2.0(+0.9 \text{ or } -0.5) \times 10^{-2}$ atoms $\text{cm}^{-2} \text{s}^{-1}$ (recalculated using the currently accepted half life⁴) measured at the Greenland site⁶. It is also 4 to 8 times smaller than the deposition rates found in most sea sediments^{9–11}. There are two possible explanations for a low ^{10}Be deposition rate at the site of our samples. First, based on measurements from bomb tests in the stratosphere, it is expected that deposition of long-lived cosmogenic isotopes at high latitudes should be substantially less than the world average⁵. In this respect, it is perhaps significant that our rate is consistent with the upper limits for the only sediment results at high latitudes¹². (Although, depending on the ^{10}Be ocean residence time, one might or might not expect such variations to be largely washed out in marine sediments due to ocean mixing.) Such an explanation would, of course, leave the Greenland⁶ result difficult to understand. Second, it is also expected that, at a given latitude, fallout rates will be roughly proportional to precipitation⁵. It will clearly be interesting to make measurements on ice samples from the coastal regions of Antarctica, where the precipitation rate is about an order of magnitude greater than at the site of the present samples, in order to determine which of these effects is dominant.

The primary objective of this study was to test whether ^{10}Be concentrations of the required sensitivity could be measured in the available ice cores (the $\sim 10^8$ atoms of ^{10}Be in each sample is several orders of magnitude smaller than could be measured by traditional beta counting, as discussed in ref. 2). In this respect the experiment was a complete success. We are thus optimistic about the chances of being able to make a complete ^{10}Be profile over the entire length of the core from which the present samples were taken (estimated to go back to 35,000 BP). If the ^{10}Be production should be as sensitive to geomagnetic field as hinted at in the present results, such a profile would offer an unparalleled opportunity for tracing the intensity variations of this field over that period. It would also allow one to look for effects such as the Laschamps–Lake Mungo–Maleifel 'events'^{8,13}, which may or may not be correlated with each other and/or with worldwide dipole variations.

The present ^{10}Be determinations in samples that were unmeasured (and unmeasurable) by any other existing technique, moves accelerator spectrometry out of the phase of exploration and into that of exploitation.

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Creep rupture at depth in a cold ice sheet

THE mechanism by which stress is released in intermediate and deep focus earthquakes and the physics of tectonic processes are of particular interest to Earth scientists. As a way of understanding such phenomena it would be valuable to have a relatively small scale tectonic system duplicating the processes taking place within the Earth. Several authors^{1–3} have speculated that processes operating within glaciers and ice sheets are analogous to those in the Earth. Experimental evidence has not supported the hypothesis of an Earth glacier equivalence, especially in terms of identifying faulting at depth within glaciers. A seismic survey made of a valley glacier in Canada⁴ found no deep ice events. However, evidence of the existence of discrete shear planes within the Antarctic Ice Sheet⁵ and evidence described here relating to the Greenland Ice Sheet, indicate that faulting takes place at depth in cold ice sheets.

At great depths beneath the surface of the Earth (500–700 km) where the pressure is of the order of 10^{10} Pa and high homologous temperatures prevail, classical laws of brittle failure do not apply. As an alternative to stress release by brittle fracture, rupture of the material may result when creep is concentrated in very narrow bands subjected to shear stresses. Consideration of the creep rupture phenomenon and its associated stress drop have led to some current models of fault mechanisms for earthquakes⁶.

The mechanism by which stress is released in intermediate and deep focus earthquakes is of particular interest to seismologists and tectonophysicists. Orowan¹ proposed that faulting at depth is initiated by the development of creep instabilities in shear layers in the Earth. The stress drop theory associated with Orowan's faulting mechanism serves as a partial base for models describing the seismic spectra from earthquakes.⁶ To verify his theory, Orowan¹ proposed that this faulting mechanism might also operate in temperate glaciers.

As a means of verifying the creep-rupture theory, Neave and Savage⁴ carried out a seismic investigation on the Athabasca Glacier in Canada. They aimed to detect shear faulting at depth within the glacier. After 390 h of seismic monitoring they concluded that creep instabilities which would be recorded as deep focus ice-quakes had not taken place in the Athabasca Glacier.

Field investigation of glaciers are generally conducted where simple flow regimes occur, so that research findings can be generalised^{7–9}. However, recent drilling at the margin of the Greenland Ice Sheet^{10,11} in a peculiar area characterised by upslope flow of cold ice over geometrically complicated bedrock (see Fig. 1), has revealed the presence of a fracture at 240 m depth in the ice. The evidence for the fracture is threefold. First, although drilling was temporarily stopped at 250 m depth surface meltwater was observed to enter the liquid-filled hole, thus showing that the drilling fluid was running out of the hole at some depth. As no crevasses, moulins or englacial conduits are present in this area, this behaviour can only indicate the existence of an open fracture that intersects the hole between the surface and 250 m depth. The hole was then completed to bedrock at 299 m. Second, following drilling the saltwater used as a drill fluid was spontaneously flushed from the upper 240 m of the hole by surface meltwater whereas the lower 59 m of the hole retained the salty water, as shown by temperature measurements made periodically after the completion of drilling¹⁰. Third, calculated temperature profiles¹² for four other

holes in the same area are in excellent agreement with measured temperatures, but the calculated profile for the hole in question agrees equally well with the measured temperatures only if the ice is assumed to flow over an area of 'dead ice' as shown in the Fig. 1. Direct observations of dead ice have been made in valley glaciers but not in cold ice sheets.

As no evidence of a pre-existing fracture was obtained, it is possible that the excess hydrostatic pressure of the drilling fluid ($\sim 2.4 \times 10^5$ Pa) and the flow shear stress caused the formation of the fracture along a zone of weakness between the dead and moving ice. Whether or not there was a pre-existing fault at this depth, the excess hydrostatic pressure caused propagation and opening of the fracture sufficient to accept a large quantity of water; hence, it is likely that the fracture propagated to bedrock around the area of dead ice (the subglacial tills are permeable in this area¹⁰). The small excess hydrostatic pressure could not have caused fracture without an existing zone of creep-enhanced weakening at this depth.

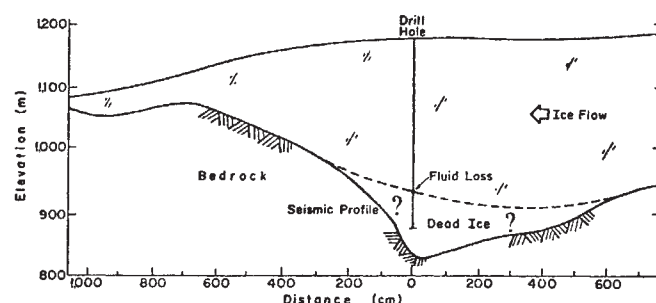


Fig. 1 Ice thickness and bedrock profile (from a seismic survey) is shown along the flow line through the drill hole, Isua, Greenland.

The geometry shown in Fig. 1 suggests an area of dead ice overridden by the ice sheet while temperature and water flow observations clearly show a fracture in the ice at 59 m above the bed. This evidence for fracture at depth in the Greenland Ice Sheet leads us to reconsider the correspondence between flow and rupture at depth in the Earth and in cold ice sheets as suggested earlier^{1,2}. If seismic evidence of deep fracturing in cold ice sheets is found we will have a large-scale tectonic system available for the study of deep fractures associated with the underthrusting of cold lithospheric plates¹³. Direct investigations at depth in ice sheets are made with relative ease as compared to the nearly impossible task of direct measurements in the Earth's mantle.

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Loss of fixed nitrogen from soils by nitric oxide exhalation

LABORATORY studies¹⁻⁸ have indicated that chemical denitrification processes in nitrogen rich soils and nitrite solutions can produce NO or NO₂ and perhaps methyl nitrite⁹. It has been suggested¹⁻⁶ that self decomposition of nitrite produces various gaseous nitrogen compounds including nitric oxide (NO) and it was thought that NO was unlikely to escape from the soil because in aerobic soils NO is readily oxidised by molecular oxygen to NO₂ and this in turn is rapidly adsorbed by soil materials and by soil water^{1,2}. However the half life of oxidation is dependent on the NO concentration because it is a termolecular reaction^{10,11}. At concentrations of 100 p.p.m. or greater (as in some studies) the half life for oxidation of NO to NO₂ by atmospheric oxygen is one hour or less respectively, whereas at low concentrations (~0.01 p.p.m.) the half life for this oxidation is of the order of 10⁴ h. This variation in the oxidation rate by molecular oxygen explains why NO at low concentrations in the field can pass unoxidised from the soil to the atmosphere and perhaps explains why in some of the laboratory studies (at high concentrations) NO₂ and not NO is detected. This NO exhalation in the field could not be detected previously due to the lack of a sufficiently sensitive measurement technique. We report here the first measurements indicating that NO is continuously exhaled from soils including ungrazed, unfertilised grassland. The measured rates of exhalation from the soil to the atmosphere are 0.06 to 0.73 × 10⁻¹¹ kg nitrogen m⁻² s⁻¹ (equivalent to 0.2 to 2.3 kg N ha⁻¹ yr⁻¹). We show that this exhalation of NO is a significant source of nitrogen oxides (NO_x) in the lower atmosphere as has been previously suggested^{12,13}. These nitrogen oxides exert a direct influence on the ambient ozone and hydroxyl radical concentrations¹⁴ which in turn pre-

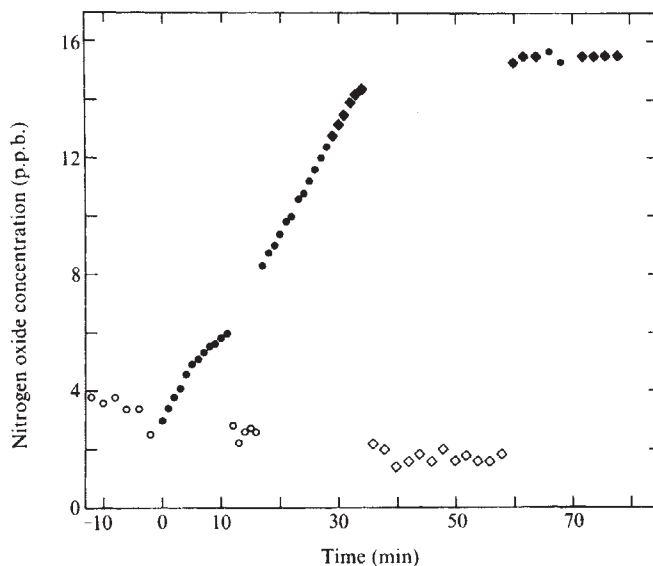


Fig. 1 Exhalation of nitrogen oxides at Aspendale on 26.11.76. The time zero corresponds to the moment of placing the box over the soil surface. ○, ambient NO_x; ◇, ambient NO; ●, box NO_x; ◆, box NO.

ester film (Dupont) to prevent gas uptake on the box walls and stirred by a 0.2 m diameter stainless steel fan driven by an external electric motor. The method will be described in detail elsewhere. NO and NO_x measurements were made with a sensitive chemiluminescent NO detector¹⁶. The NO_x measurement, (obtained by diverting the sample flow through a commercial molybdenum converter) gives the total gaseous concentration of NO, NO₂, alkyl nitrites and nitrates and peroxyacetylnitrate. These are accepted methods for measuring low concentrations of nitrogen oxides in ambient air^{18,19}.

Measurements were made at three locations described briefly in Table 1 and in more detail elsewhere²⁰⁻²². The range of conditions covered included before and after rain, day and night and spring and autumn. On every occasion measurable exhalation was found. In all experiments there was no significant difference between the concentration of NO and NO_x in the box, indicating that NO is the dominant species exhaled from the soils studied.

Kim²⁴ measured NO₂ liberation from forest soils in Korea with a closed system consisting of a sealed plastic hood containing 0.01 M sodium hydroxide absorbent. The sodium hydroxide solution used by Kim also collects NO rather inefficiently²⁵ and his results, therefore, possibly need reconsideration. Also the presence of this absorbent can inhibit autotrophic nitrification of ammonium by removing carbon dioxide²⁶.

A typical set of results is shown in Fig. 1. Immediately after placing the box over the surface there was a monotonic increase in the NO in the box above ambient levels. Generally, the concentration in the box gradually approached a maximum or equilibrium value. This behaviour indicates that there is some competing uptake mechanism or back-reaction that, in conjunction with the exhalation process, regulates the concen-

Table 1 Description of experimental sites

Location	Land usage	Soil description ²³	Nitrogen fertiliser usage
Aspendale (38°S)	Meteorological observation area with mown grass	Siliceous sand with minimal development pH 6.2-7.1	None
Edithvale (38°S)	Dairy cattle grazing area	Peaty soil pH 6.7 (one measurement)	None
Conargo (35°S)	Site 1: disused cattle milking yard	Hard setting loam soil with	1 None
	Site 2: irrigated pasture	red clay subsoil	2 None in previous 4 years
	Site 3: sheep grazing —no pasture improvement	pH 6.5-7.6	3 None

dominantly determine the oxidation rate of almost all substances in the troposphere.

We determined the NO and NO_x flux from various soil surfaces by placing an open-ended box over the soil and measuring the rate of increase in concentration of these gases in the box during the following few minutes. It is assumed that the presence of the box during this time did not impede or exaggerate the gaseous flux from the soil. The continuing increase of NO in the box after sampling is discontinued indicates that there is no artificial pressure-induced exhalation of soil air into the box. The box, similar to that described elsewhere¹⁵, was a perspex cube (0.8 m per side), internally lined with Mylar poly-

Table 2 Soil analyses at Conargo sites

Site	Soil moisture (%)	Soil pH	NO ₃ -N p.p.m.	NH ₄ -N p.p.m.
1	18.6	7.6	2.1	4.0
2	10.7	6.5	1.3	3.8
3	12.2	7.2	13.1	2.4

tration of NO in the box bringing it into equilibrium with the soil/soil-atmosphere system. Concentrations of NO greater than the equilibrium value were artificially injected into the box on some occasions. The NO concentration then always decreased back to the same equilibrium value for the site.

The soil analyses presented in Table 2 (made at the time of the exhalation experiments) show that the mechanism for NO production in soil operates at a soil pH near 7 and at the p.p.m. nitrate-N and ammonium-N levels. Analyses performed recently show similar concentrations at the Aspendale site; these analyses also showed less than 0.2 p.p.m. nitrite-N. Nitrite analyses were not performed at the time of the experiments. Our results confirm laboratory experiments^{3-5,8} where detection of volatilisation products as either NO or NO₂ occurred for neutral and slightly alkaline as well as for acidic soils.

Our experimental data and the calculated emission rates are shown in Table 3. If the average flux of 0.3×10^{-11} kg N m⁻² s⁻¹ is integrated over a year then a figure of 1 kg N ha⁻¹ yr⁻¹ is obtained.

The fluxes observed for ungrazed and grazed pastures are 0.16 and 0.35×10^{-11} kg m⁻² s⁻¹ respectively. If these are representative of globally averaged land emission rates then a

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Table 3 Experimental details and results

Location and date	Local time	NO flux (10^{-11} kg (N) m ⁻² s ⁻¹)	NO ₂ * flux (% of NO flux)	Rainfall in previous 24 h(mm)	Air temp. (°C)
Edithvale					
16.11.76	1140	0.22	<3%	5.0	16
17.11.76	1100	0.59	<5%	3.2	14
18.11.76	1130	0.35	<3%	3.4	14
Aspendale					
22.11.76	1700	0.06	—	0	15
23.11.76	Daytime	0.15	<3%	0	16
26.11.76	1130	0.26	<2%	0.4	19
Conargo					
Site 1					
16.5.77	1600	0.37	<2%	0	16
17.5.77	0800	0.15	<3%	0	13
Site 2					
17.5.77	1530	0.25	<3%	0	~16
Site 3					
19.5.77	0730	0.25	<8%†	7	14
19.5.77	2130	0.26	<2%	0	15
20.5.77	1030	0.73	<2%	0	~16

* Includes also alkyl nitrates, nitrites and PAN.

† The uncertainty in this measurement is large because of the presence of NO₂ in the chamber (from ambient air) at the start of the experiment.

global annual value of the order of 10^{10} kg N yr⁻¹ is obtained which can then be compared to the global nitrogen fixation rate of 2 to 3×10^{11} kg N yr⁻¹ (ref. 27). The loss of NO from the soil is not a permanent loss of fixed nitrogen as this nitrogen is most probably returned to the soil as nitrates in rainwater. Thus NO exhalation may be a significant process in redistributing fixed nitrogen throughout the global biosphere.

In the Northern Hemisphere troposphere these annual emissions may be 0.8×10^{10} kg N which, with the anthropogenic NO_x sources of 1.5×10^{10} kg N, are sufficient to account for the nitrate deposition in rainwater of 2×10^{10} kg N (ref. 13).

The emissions reported here are, per unit area, about 40 times larger than the depositions of NO_x nitrogen reported for Antarctic ice by Parker *et al.*^{28,29}. We suggest the NO_x nitrogen in Antarctic ice may result from the long range transport of fixed nitrogen from the continental regions of the Southern Hemisphere.

Coaxial pure shear in Jamaican blueschists and deformation associated with subduction

A RECENT report of deformation features in high pressure-low temperature blueschists on the Ile de Groix, France, has demonstrated that the style of deformation is one of progressive, non-coaxial simple shear¹. I present here evidence that the blueschists in Jamaica underwent a coaxial pure shear deformation contemporaneous with metamorphism and suggest that simple shear deformation in blueschists is no more compatible with subduction than any other deformation style. I suggest that domains of relatively homogeneous deformation occupying tens of km³ may develop in subduction complexes.

Metamorphic rocks in Jamaica consist of a narrow, NW-SE trending belt of metagreywackes and metabasites metamorphosed in blueschist, greenschist and amphibolite facies conditions^{2,3}. The belt is situated along the southern edge of the Blue Mountain massif in eastern Jamaica and the blueschists lie at the eastern end of the belt about 30 km ENE of the city of Kingston (for exact location see ref. 3). The metamorphism was the result of subduction which probably occurred in the late Cretaceous^{2,3}, but the only isotopic ages available are from biotite and hornblende in the amphibolite facies rocks⁴. The blue amphibole crossite occurs in the eastern extremity of the belt and is associated with pumpellyite, stilpnomelane, albite, clinozoisite and sphene. All CaCO₃ identified so far is calcite. Lawsonite does not seem to occur, either because the pressure/temperature gradient was not sufficiently high or because of high CO₂ pressure during metamorphism⁵. Some rocks are not entirely recrystallised and contain relict igneous augite.

The blueschists typically show an *L-S* tectonite fabric which plunges approximately 30° to the NNW. This orientation is not

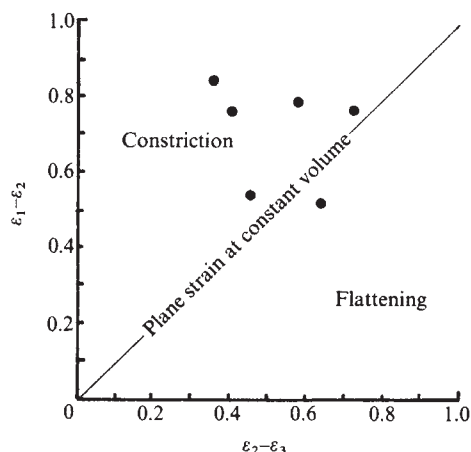


Fig. 1 Logarithmic strain plot of finite strains (ϵ) from L-S tectonites of Jamaican blueschist terrain. Most fall in the field of constriction.

consistent because the rocks have undergone further deformation associated with Tertiary E-W left lateral motion on the northern Caribbean plate boundary which lies to the north of Jamaica. The lineation itself is due to stretching parallel to the x-axis of the finite strain ellipsoid. Ellipsoidal, microcrystalline sphene blebs are probably derived from alteration of original ilmenite. A statistical study of these in individual specimens^{6,7} provides finite strain estimates, most of which fall in the field of constriction of Fig. 1.

Evidence for the coaxial nature of the strain history is exhibited by the relict augites which have undergone paracrystalline microboudinage. Amphibole fibres which have grown epitaxially between boudins are zoned continuously from riebeckitic actinolite to crossite (Fig. 2). The x-axis of the finite strain ellipsoid, amphibole fibres and elongation as defined by the boudinage are all parallel. These textural features are not unique to Jamaica and have been reported from Washington, US^{8,9}.

With continuing strain either the material properties of the rock changed or there was a change in the rate of strain, as further strain was taken up heterogeneously by the formation of discoidal ductile 'fractures' or tears. The planes of the tears are perpendicular or subperpendicular to x and are filled with tremolite and quartz fibres parallel to x. Tears are up to 0.1 m in thickness and 0.4 m in diameter and seem to be distributed randomly through the rock at intervals from a few centimetres to

tens of centimetres. They have contributed to a further 5–10% extension in the x direction. This pattern of deformation is found throughout the entire blueschist terrain (about 12 km²).

The progressive coaxial pure shear reported here contrasts with the progressive simple shear reported from the Ile de Groix¹. Both these modes of deformation were contemporaneous with high pressure–low temperature metamorphism which most workers believe is produced in the crustal portions of subduction zones^{10,11}. On the megascopic scale the regional pattern of deformation is one of simple shear as the lower plate moves under the upper. However, on a finer scale, deformation is likely to be more heterogeneous. Regimes of simple shear may well exist in the rocks overlying the downgoing plate, but there is no evidence to suggest that they persist throughout the whole accretionary prism. Consideration of the irregularities in the topography of the downgoing oceanic plate and variations in the geometry of the subduction zone along strike suggest that both the simple shear of the Ile de Groix rocks and the pure shear of the Jamaican rocks are special cases and that most syntectonic metamorphic deformation in blueschist terrains is likely to be heterogeneous at least on a regional scale. The relatively homogeneous but different styles of deformation developed in the Jamaican and Ile de Groix terrains are present in a volume of rock of tens of km³. Do deformation domains on this scale develop in subduction complexes, or is heterogeneity developed on a finer scale in other blueschist terrains? Mechanisms of blueschist emplacement ('obduction') will be equally varied and complex. Thus, many patterns of strain in blueschists may be compatible with subduction and emplacement. More comparative studies are needed before general statements can be made.

I thank Geoff Wadge for his constructive criticism.

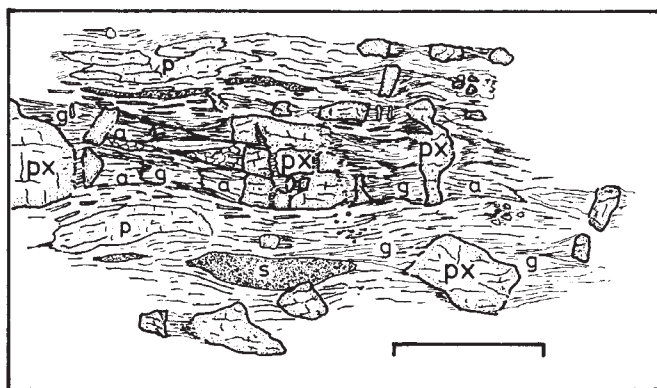
GRENVILLE DRAPER

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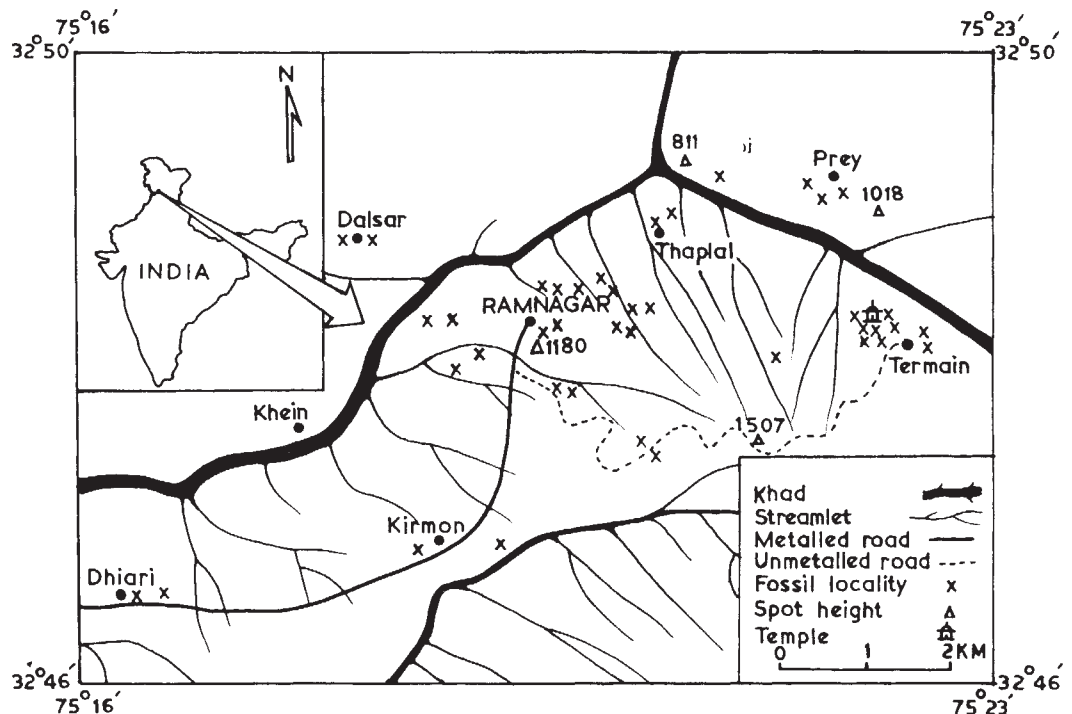
Fig. 2 Sketch from thin section cut parallel to x-z plane of finite strain ellipsoid, showing typical microboudinage texture developed in metabasites. px, relict augite; g, glaucophane/crossite; a, riebeckitic actinolite; p, pumpellyite; s, ellipsoidal sphene blebs. Scale bar, 1 mm.



Geology, fauna and palaeoenvironments of Lower Sivalik deposits around Ramnagar, India

ALTHOUGH the Sivalik deposits of the Ramnagar basin (Jammu and Kashmir, India) have been known to palaeontologists and palaeoanthropologists for about 100 yr, little has been reported about the fauna, flora and palaeoenvironments of the region. Most reports centre round the primates^{1–2}, and no attempt has been made to report the other vertebrates in detail. We surveyed the Ramnagar area during the field seasons of 1975–77, and catalogued more than 600 fossil specimens representing a diversity of mammals, reptiles and fish, recovered from several localities shown in Fig. 1. We report those finds here.

The Ramnagar sediments contain fauna characteristic of the Chinji Formation of the Lower Sivaliks. These deposits can be correlated biostratigraphically with the Vindobanian of Europe and possibly the Fort Ternan deposits of East Africa³ which have been assigned a potassium-argon date of 12–14 Myr. No radiometric dates are available for the Chinji deposits at Ramnagar but they can be assigned an age equivalent to 11–13 Myr on the basis of their fauna.

Fig. 1 Generalised locality map of Ramnagar.

The outcrops at Ramnagar show a remarkable uniformity in lithology. The beds, which are best exposed along streamlets and road cuttings, consist of alternate layers of argillaceous and arenaceous sediments, the latter dominating the former. The

Table 1 Fossil vertebrates from Ramnagar

Pisces	
Siluridae	indet.*
Reptilia	
Chelonia	<i>Trionyx</i> sp.
	<i>Lissemys</i> sp.
Crocodylia	<i>Crocodylus</i> sp.
	<i>Gharialis</i> sp.
Mammalia	
Primates	
Hominidae	<i>Ramapithecus punjabicus</i>
Rodentia	
Rhizomyidae	<i>Rhizomyoides sivalensis</i> *
	<i>Rhizomyoides punjabiensis</i> *
Ctenodactylidae	<i>Sayimys sivalensis</i> *
Muridae	indet.
Carnivora	
Creodonta	<i>Dissopsalis</i> sp.*
Fissipeda	<i>Vishnufelis</i> sp.*
Amphicyonidae	<i>Amphicyon</i> sp.*
Lutrinae	indet.
Proboscidea	
Gomphotheriidae	<i>Gomphotherium</i> sp.*
Deinotheriidae	<i>Deinotherium pentapotamiae</i> *
Perissodactyla	
Rhinocerotidae	<i>Caementodon</i> sp.*
	<i>Chilotherium?</i> <i>intermedium</i> *
	<i>Brachypotherium</i> sp.*
	<i>Aceratherium perimense</i> *
Artiodactyla	
Suidae	<i>Listriodon pentapotamiae</i>
	<i>Conohyus chinjiensis</i>
Anthracotheriidae	<i>Anthracotherium punjabiense</i> *
Tragulidae	<i>Dorcabune anthracotheroides</i> *
	<i>Dorcabune hyaemoschoides</i> *
	<i>Dorcatherium majus</i>
	<i>Dorcatherium minus</i>
Giraffidae	<i>Giraffokeryx punjabiensis</i>
Bovidae	<i>Protragocerus</i> sp.*
	<i>Miotragocerus</i> sp.*
	<i>Gazella</i> sp.*

* This is the first report from Ramnagar.

clayey units are greenish to dark pinkish and show evidence of nodular weathering. The sandstones contain a high percentage of undecomposed rock fragments and are frequently cross-bedded. The upper surface of sandstones is marked with asymmetrical ripples and a network of desiccation fissures in many places.

During the survey numerous fossilised fragments of various mammals were collected (Table 1). Detailed descriptions of the fossils are not available and the identifications should be considered provisional, although in broad outline they are likely to stand. In addition to primates a diversity of proboscideans, perissodactyls and artiodactyls were found. No specimen of *Hipparion* was collected and among the rodents, apart from a few rhizomyoids, only a single tooth of a ctenodactylid and a partial skull of a murid were recovered.

The general nature of the sediments is indicative of deposition in a flood-plain. The presence of red and brownish coloration in many rock units suggests that a considerable portion of sediments was deposited in oxidising and possibly warm and humid conditions. Intermittent desiccation is indicated by the occurrence of desiccation fissures on the surface of arenaceous units. The common occurrence of undecomposed rock fragments in sandstones implies short or rapid transportation, which in turn implies deposition of the sediments close to the source area.

The mammalian fossils consist mainly of suids, small artiodactyls and primates. Their abundance provides indirect evidence for the existence of general forested conditions in the vicinity of the depositional basin. The deinotheres and primitive gomphotherids are thought to have lived on soft vegetation and may have inhabited areas adjacent to water bodies and abounding in succulent and fleshy plants. The abundance of tragulids like *Dorcatherium* and *Dorcabune* implies the presence of bushy forest undergrowth near the site of deposition.

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Natural source of tetraalkyllead in air

THE biological methylation of inorganic mercury in sediments in both aerobic and anaerobic conditions is well known¹, and attention has focussed on the possibility of a similar process for lead in the environment. It has been possible to methylate lead in laboratory studies, with the resultant evolution of gaseous tetramethyllead²⁻⁴, but there is still considerable doubt as to whether such processes occur in the environment⁵. A report⁶ that tetraalkyllead accounts for 10–24% of the total lead in the liver of fresh and frozen samples of cod and 39% of the total lead in mackerel muscle lends credence to the possibility. However, we can now present firm evidence of a natural source contributing tetraalkyllead to the atmosphere.

Our evidence was obtained from a monitoring programme carried out between August 1977 and January 1978, during which we determined 24-h average concentrations of particulate lead and gaseous tetraalkyllead in duplicated air samples collected on 24 separate days, and 48-h averages obtained on four occasions, at three remote rural sites around Lancaster selected because of their freedom from local vehicular pollution sources. Particulate lead was collected on a 0.45- μm membrane filter (Millipore type HAWP 04700) and determined by flameless atomic absorption spectrometry after extraction into acid solution⁷. Tetraalkyllead was trapped quantitatively downstream of the filter in iodine monochloride solution and the lead concentration was determined by flameless atomic absorption spectrometry after selective extraction into acid solution, by means of a technique specific for tetraalkyllead compounds^{8,9}.

To interpret our results (Table 1) it is necessary to consider the known sources of particulate lead and tetraalkyllead in rural air. Furthermore, while the data can be examined in terms of their absolute concentration, it is of more value to consider the concentrations of tetraalkyllead as a percentage of total lead, as these figures depend less on site characteristics and meteorological conditions and facilitate comparison between data¹⁰. Particulate lead and tetraalkyllead in the atmosphere are due principally to emissions from vehicles fuelled by leaded petrol. These emissions arise predominantly in urban areas. Harrison and Perry¹⁰ have reviewed available data on tetraalkyllead concentrations in these urban source areas (Table 1). High concentrations of tetraalkyllead can develop around petrol stations and in underground car parks, where there is considerable evaporative loss of petrol. However, tetraalkyllead as measured by reliable techniques is more typically present at lower concentrations in urban air, representing only a small proportion of

total lead, usually about 1–4%. We made further measurements of tetraalkyllead in air, finding it to represent 4.5–5.7% of total lead close to a busy street in Lancaster city centre, when an activated carbon trap method was used,¹¹ and 0.22–0.46% of total lead alongside a remote section of the M6 motorway in Cumbria, measured as 24-h averages by the sampling and analytical method⁹. Thus we conclude from the available data that in the absence of a strong local source tetraalkyllead is most likely to represent 1–4% of total lead in urban air with an overall range of 0.5–8%.

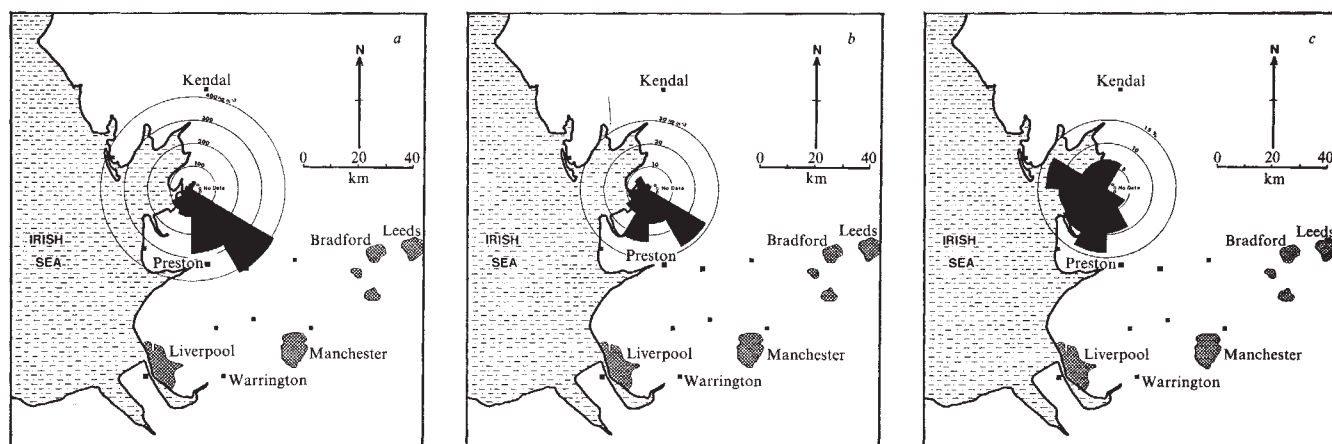
Table 1 Concentrations of particulate lead and tetraalkyllead in urban and rural air

	Particulate lead (ng m^{-3})	Tetra- alkyllead (ng m^{-3})	Ratio tetra- alkyllead/ total lead (%)	Source
Urban air (typical values)	500–14,500	<6–206	0.3–4	ref. 10
Petrol station	5,500–6,000	210–590	4–10	ref. 10
Underground car park	10,800–12,200	1,800–2,200	15–18	ref. 10
Rural air	10–660	0.5–230	1.5–33	This work

Lead pollution of rural air arises mainly from advection of urban pollution, and the concentrations of particulate lead and tetraalkyllead in rural air due to urban emissions depend on atmospheric dispersion and dilution effects, the distance of the urban source from the rural site, and the extent of losses *en route*. Only the latter, however, affects the relationship between particulate lead and tetraalkyllead concentrations. It is well known that a large proportion of particulate lead emission is within the size range of particles which form a stable aerosol, so that this lead is lost only slowly from the atmosphere, with a mean lifetime probably in the range 1–7 d.¹² We have determined the mechanism of tetraalkyllead breakdown in the atmosphere¹³ and can predict that tetramethyllead, the predominant tetraalkyllead compound in the atmosphere¹⁰, will have a typical mean lifetime of the order of several days. The loss of both particulate lead and tetraalkyllead from the atmosphere will therefore occur at similar rates, with a resultant ratio of tetraalkyllead to total lead predicted for a rural site of close to the original urban value of 1–4%.

Our data, summarised in Table 1, show that there is a significant departure from expected values. The concentration range for particulate lead, as anticipated, lies considerably below that in urban areas, while the range for tetraalkyllead does not differ so greatly. Indeed, the peak rural concentration of 0.23 $\mu\text{g m}^{-3}$ tetraalkyllead is rarely exceeded in street air measurements¹⁰. Furthermore, seven out of the 28 samples

Fig. 1 Time-weighted pollution roses for lead concentrations measured at three rural sites around Lancaster. *a*, Particulate lead; *b*, tetraalkyllead; *c*, percentage ratio tetraalkyllead/total lead.



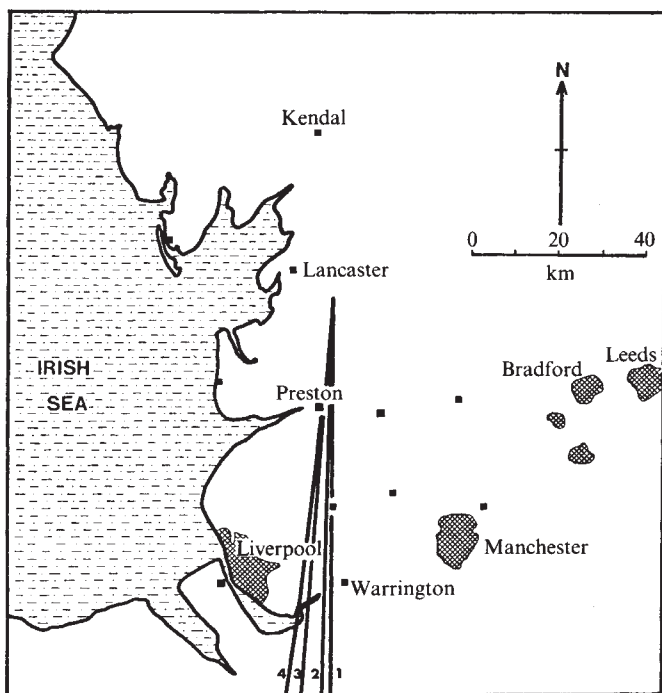
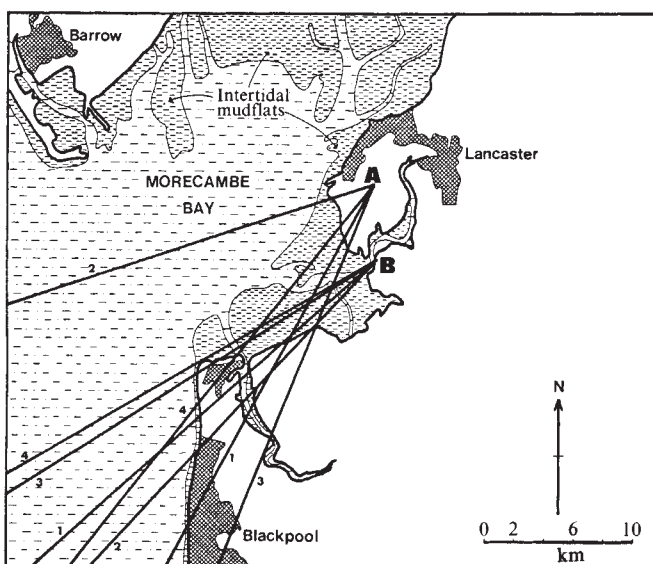


Fig. 2 Terminal directions of geostrophic backward air mass trajectories for a sample period with a tetraalkyllead/total lead ratio of 1.5%. Time of arrival of air mass: 1, 1800 h 18/10/77; 2, 0000 h 19/10/77; 3, 0600 h 19/10/77; 4, 1200 h 19/10/77.

revealed unusually high tetraalkyllead to total lead percentages, in excess of 15%, with a maximum of 33%. Such high percentages are most unlikely to arise from urban emissions. We therefore examined our data in more detail, to identify any relationship between the concentrations and wind direction during a sampling period. Time-weighted pollution roses were constructed, based on hourly wind direction frequency data at 10 m height, divided into 30° sectors (Fig. 1). These reveal the expected pattern for particulate lead, with evidence of the strong influence of urban emissions to the south and south-east of the

Fig. 3 Terminal directions of geostrophic backward air mass trajectories for sample periods with a tetraalkyllead/total lead ratio in excess of 20%. A, Ratio of tetraalkyllead/total lead = 25%. Time of arrival of air mass: 1, 1200 h 30/8/77; 2, 1800 h 30/8/77; 3, 0000 h 31/8/77; 4, 0600 h 31/8/77. B, Ratio of tetraalkyllead/total lead = 27%. Time of arrival of air mass: 1, 1800 h 16/12/77; 2, 0000 h 17/12/77; 3, 0600 h 17/12/77; 4, 1200 h 17/12/77.



sampling sites (Fig. 1a). The picture that emerges for tetraalkyllead, on the other hand, is far less clear (Fig. 1b). When, however, the data for the percentage of tetraalkyllead in total lead are examined a distinct directional influence is revealed (Fig. 1c). High mean sector values of 10–15% are associated with winds of 180–300°, while lower mean values of 5–6% occur with winds from other quarters. These data indicate that a widespread source of tetraalkyllead emissions lies generally to the west of the sampling sites.

Because wind directions indicate only local low-level air movements, geostrophic backward air mass trajectories have also been constructed at 6-h intervals during selected 24-h sample periods (that is, four trajectories per sample), to show synoptic scale air movements. The lowest individual percentage values measured of about 2% are associated with air which had passed over the urban areas to the south and south-east (Fig. 2).

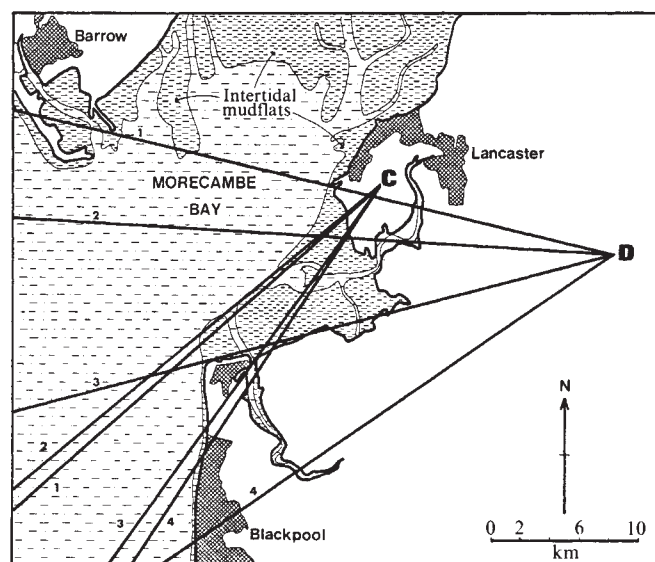


Fig. 4 Terminal directions of geostrophic backward air mass trajectories for sample periods with a tetraalkyllead/total lead ratio in excess of 20%. C, Ratio of tetraalkyllead/total lead = 21%. Time of arrival of air mass: 1, 1200 h 31/8/77; 2, 1800 h 31/8/77; 3, 0000 h 1/9/77; 4, 0600 h 1/9/77. D, Ratio of tetraalkyllead/total lead = 33%. Time of arrival of air mass: 1, 1800 h 31/10/77; 2, 0000 h 1/11/77; 3, 0600 h 1/11/77; 4, 1200 h 1/11/77.

These values are consistent with those to be expected in an urban plume largely unaffected by losses of either particulate lead or tetraalkyllead. On the other hand, periods during which values exceeded 20% are all associated with air which had passed over open sea and the coastal areas adjacent to the sampling sites (Figs 3 and 4). Further examination of our data leads us to suggest that the source of tetraalkyllead is more likely to be associated with the extensive coastal/estuarine intertidal mud flats to the west of our sampling sites, rather than the open sea. Both seawater and sediments in this region have been shown to have elevated levels of inorganic lead (ref. 14 and S. R. Aston personal communication), presumably arising from estuarine discharges of anthropogenic origin. In our view, this source of tetraalkyllead can only be explained by a natural environmental methylation of lead.

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Light-dependent net production of carbon monoxide by plants

PLANTS take up ^{14}CO and incorporate it into organic compounds in the light and in the dark, and it has been suggested that this decreases the concentration of CO polluting the atmosphere^{1,2}. Measurements of ^{14}CO uptake, however, have excluded any concomitant release of CO by the plants and are insufficient for conclusions to be drawn on net CO exchange. Many organisms (microbia, plants, fungi and animals, including man) can produce CO (refs 3–5). We report here that using a Siemens U 180 process gas chromatograph, we have observed a light-dependent net production of CO by intact plant shoots which seems to be linked to photorespiration.

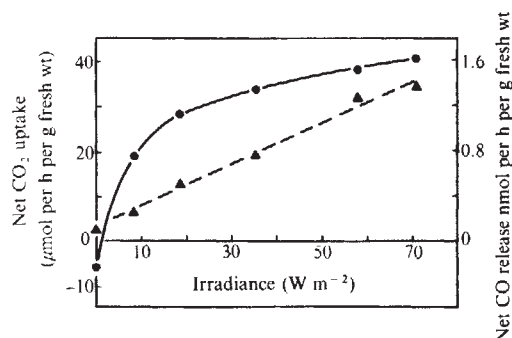


Fig. 1 Dependence of CO (Δ) and CO_2 ($\bullet$) exchange on irradiance. Leaf temperature 28.3°C ; CO concentration in the gas stream supplied to the gas-exchange chamber $2.07 \text{ mm}^3 \text{ m}^{-3}$, average CO concentration within the chamber during the experiment $4.78 \text{ mm}^3 \text{ m}^{-3}$; CO_2 concentration $250 \text{ cm}^3 \text{ m}^{-3}$; O_2 concentration 21 vol %.

In two sets of experiments the shoot of an intact plant of *Nerium oleander* L. with a total leaf surface area of 0.07 m^2 and 0.18 m^2 , respectively, was sealed in a gas exchange chamber with a volume of 0.01 m^3 . (A leaf surface of 0.0065 m^2 is equivalent to 1 g fresh weight. Similar responses are observed with *Vicia faba* L.). Light was obtained from two 400-W HRIL phosphorus-coated metal halide lamps. Various mixtures of gases were passed through the chamber at $30 \times 10^{-6} \text{ m}^3 \text{ s}^{-1}$. Net CO exchange was measured with the gas chromatograph and net CO_2 exchange with an URAS 2T infrared gas analyser. The mixtures were obtained by mixing O_2 , N_2 , CO_2 and synthetic air from gas cylinders, using dosage pumps and gas flow meters. Traces of CO were removed by passing the gas stream over granulated CO catalyser (Dräger). When required, different concentrations of CO were obtained by adding to the CO-free mixtures various amounts of normal laboratory air whose CO concentration was known from analysis with the gas chromatograph. The atmosphere at our laboratory, at the periphery of the eastern part of Darmstadt, contained up to $3 \text{ cm}^3 \text{ m}^{-3}$ CO at peak traffic hours and $0.3 \text{ cm}^3 \text{ m}^{-3}$ at other times of the day. This CO caused some problems because of temperature-dependent diffusion of CO into the gas-exchange chamber through small but unavoidable leaks in the apparatus. This diffusion was 1.9 nmol h^{-1} at 16.6°C , 3 nmol h^{-1} at 22.5°C and 13.8 nmol h^{-1} at 39.2°C . Corrections for this contamination were made in calculating net CO production by the plant.

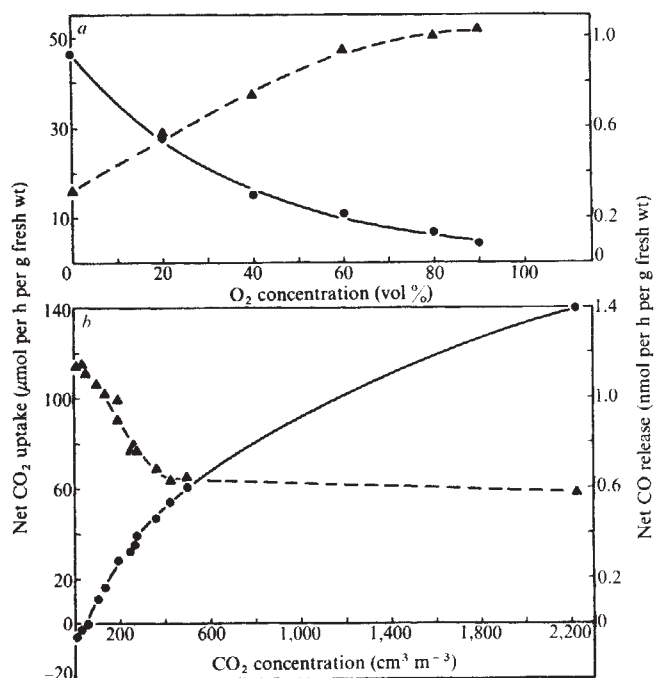
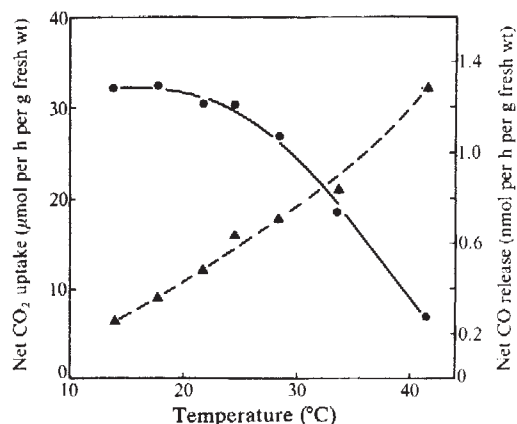


Fig. 2 *a*, Dependence of CO (Δ) and CO_2 ($\bullet$) exchange on O_2 concentration. Leaf temperature 27.5°C ; CO concentration supplied to the gas-exchange chamber $1.61 \text{ mm}^3 \text{ m}^{-3}$, average CO concentration within the chamber $4.63 \text{ mm}^3 \text{ m}^{-3}$; CO_2 concentration $275 \text{ cm}^3 \text{ m}^{-3}$; irradiance 28.0 W m^{-2} . *b*, Dependence of CO and CO_2 exchange on CO_2 concentration. Leaf temperature 25.5°C ; CO concentration supplied to the gas-exchange chamber $1.9 \text{ mm}^3 \text{ m}^{-3}$, average CO concentration within the chamber $4.45 \text{ mm}^3 \text{ m}^{-3}$; O_2 concentration 21 vol %; irradiance 35.8 W m^{-2} .

When the atmosphere in the chamber contained 40 – $825 \text{ mm}^3 \text{ m}^{-3}$ CO the *N. oleander* leaves consistently increased the CO concentration by 10 – $17 \text{ mm}^3 \text{ m}^{-3}$. When the atmosphere contained even 100 – $120 \text{ cm}^3 \text{ m}^{-3}$ CO, plants still did not show a net absorption of CO (ref. 6).

Net production of CO was then studied with low concentrations of CO in the gas stream supplied to the chamber ($\sim 2 \text{ mm}^3 \text{ m}^{-3}$) and compared with CO_2 gas exchange. CO production was stimulated considerably by light; there seemed to be a small insignificant release of CO in the dark (Fig. 1). Production of CO in the light was increased by increasing the

Fig. 3 Dependence of CO (Δ) and CO_2 ($\bullet$) exchange on air temperature in the gas-exchange chamber. CO concentration supplied to the gas-exchange chamber $2.02 \text{ mm}^3 \text{ m}^{-3}$, average CO concentration within the chamber $4.54 \text{ mm}^3 \text{ m}^{-3}$; CO_2 concentration $250 \text{ cm}^3 \text{ m}^{-3}$; O_2 concentration 21 vol %; irradiance 18.7 W m^{-2} .



concentration of O_2 and decreased by increasing the concentration of CO_2 in the ambient atmosphere (Fig. 2). Photosynthetic CO_2 fixation showed an inverse relationship to the concentrations of CO_2 and O_2 (Fig. 2). Increasing temperature greatly enhanced CO release (Fig. 3).

As photorespiration responds in a similar way to environmental parameters⁷⁻⁹, these results suggest that light-dependent production of CO is a by-product of photorespiratory C_1 metabolism. CO could be derived from the glycolate pathway of photorespiration in two ways. First, direct oxidative decarboxylation of glycolate may produce formate¹⁰ and hence CO . Second, formate and CO may originate from the tetrahydrofolate activated C_1 unit formed during photorespiratory synthesis of one molecule of serine from two molecules of glycine¹¹. The significance of this minimal production of CO is not clear; nonetheless, the net exchange of CO by plants is a small emission, not absorption.

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Photorespiratory nitrogen cycle

MOST temperate plants form phosphoglyceric acid as the first stable product of photosynthesis. These plants also photorespire, that is, during CO_2 fixation they simultaneously release CO_2 to the atmosphere from the oxidation of an intermediate of the photosynthetic process^{1,2}. The reaction below, which takes place in the mitochondria^{3,4}, is chiefly responsible for this CO_2 release.



Photorespiration is considered wasteful because it loses CO_2 and energy, but little emphasis has been placed on the simultaneous and stoichiometric release of NH_3 . Based on estimates of photorespiration in wheat leaves of $80 \mu\text{mol } CO_2 \text{ h}^{-1} \text{ g}^{-1}$ fresh weight^{5,6}, NH_3 loss far exceeds the calculated rates of primary NH_3 assimilation from NO_3^- reduction⁷⁻⁹. This NH_3 must be reassimilated if the plant is not to lose all its organic nitrogen. Recent ^{15}N studies show that NH_3 is recycled¹⁰ but it is not known if reassimilation is catalysed by glutamine synthetase (GS), as is primary NH_3 assimilation^{11,12}, or by glutamate dehydrogenase (GDH) also present in the mitochondria^{11,12}. Identification of the pathway is important because it determines the extent to which NH_3 cycling further increases the energy loss caused by photorespiration. We report here experiments which show that reassimilation involves GS and we propose a series of reactions which make up the photorespiratory nitrogen cycle.

An estimate of the amount of GS and GDH in wheat leaves was made. The values obtained, in nKat per g fresh weight, of 40 for GS and of 54 for GDH indicate that both enzymes may be present in sufficient amounts to catalyse NH_3 reassimilation despite the high K_m of GDH for NH_3 .

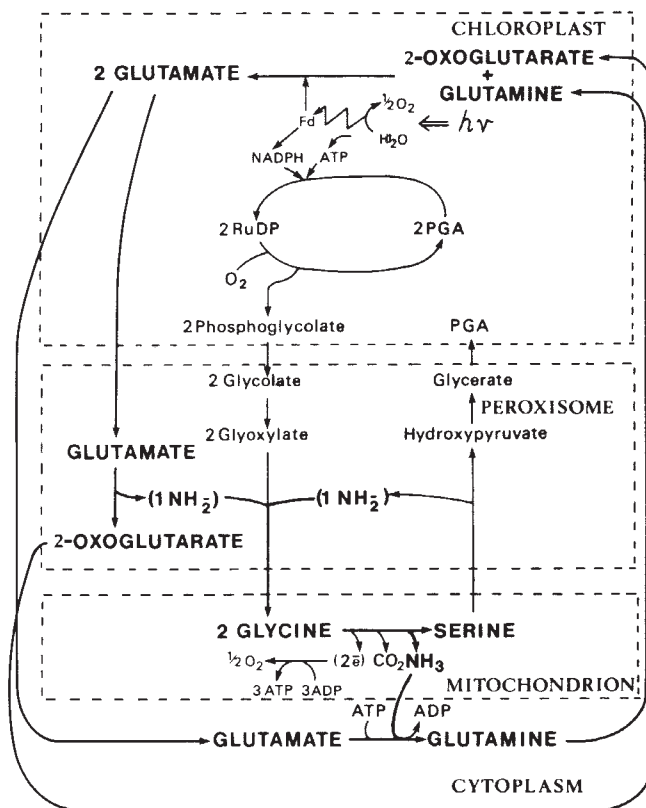


Fig. 1 The photorespiratory nitrogen cycle (the complex stoichiometry of the Calvin cycle is not included).

To study the loss of NH_3 and its reassimilation *in vitro*, mitochondria were isolated from spinach leaves by the method of Douce *et al.*¹³. They were shown to produce $^{14}CO_2$ and $^{15}NH_3$ in about equal amounts from $[^{15}N, 1-^{14}C]$ -glycine (Table 1); serine was also produced as found previously with tobacco³ and spinach¹⁴ mitochondria. The mitochondrial preparations contained substantial amounts of GDH (about a third of the total in the original homogenate) and small amounts of GS ($\leq 0.2\%$); this GS was separated from the mitochondrial marker enzymes when the preparations were centrifuged on a sucrose density gradient. To test if the NH_3 produced by the mitochondria could be reassimilated *in situ* the various reaction components required for GDH or GS activity were added to the mitochondrial incubations (Table 1, treatments 2-4); there was no decrease in the amount of NH_3 evolved or in the ratio of $^{15}NH_3/^{14}CO_2$ released. These results, and those of Woo and Osmond¹⁴ showing that NH_3 is not retained within the mitochondria, suggest that reassimilation occurs outside.

Recent experiments have shown that GS is almost equally distributed between the chloroplast and the cytosol¹⁵; the cytosol GS, present immediately external to the mitochondria, might well function in reassimilation. To test this, chloroplasts were removed from an homogenate of pea leaves by centrifugation¹⁶. The GS was purified 100-fold from the supernatant liquid by ammonium sulphate precipitation and sequential chromatography on Sephadex G-25, DEAE-cellulose and Sepharose 4-B. Preparations, with a minimum specific activity of 111 nKat per mg protein as measured by the γ -glutamyl synthetase assay¹⁷, were added to reaction mixtures also containing mitochondria. Difficulties were experienced in combining the two systems, as the requirements of each for maximum activity differ - the conditions indicated in Table 1 represent a compromise. In the presence of GS little NH_3 was recovered from the reaction medium, indicating that it had been reassimilated, but there was increased release of $^{14}CO_2$ and the ratio of $^{15}NH_3/^{14}CO_2$ dropped to 0.13. The increase in CO_2 release could be explained by a faster rate of electron transport

Table 1 Release of NH₃ and CO₂ from [¹⁴C, ¹⁵N]-glycine by mitochondria

Treatment	Reaction medium	CO ₂ released from glycine (μmol)	NH ₃ released from glycine (μmol)	Total NH ₃ released (μmol)	Ratio of ¹⁵ NH ₃ : ¹⁴ CO ₂
1	Control	0.623	0.633	0.870	1.01
2	Control + 2-oxoglutarate	0.621		0.875	
3	Control + glutamate	0.615		0.945	
4	Control + 2-oxoglutarate, glutamate and oxaloacetate	0.847	0.915	1.182	1.08
5	Control	1.04	1.15	1.31	1.10
6	Control + GS	1.85	0.24	0.35	0.13

Treatments 1–4 control contained, 3.3 mM ATP, 16.4 mM (1-¹⁴C, ¹⁵N)-glycine (0.05 mCi per mmol 99 atom % ¹⁵N), 246 mM mannitol, 4.1 mM MgCl₂, 8.2 mM KCl, 8.2 mM KH₂PO₄, 0.08% bovine serum albumin, mitochondria from market spinach containing 0.8 mg protein and, where indicated, 16.4 mM glutamate, 16.4 mM 2-oxoglutarate, 16.4 mM oxaloacetate. The final volume (0.61 ml) was incubated at pH 7.2 for 20 min at 22 °C. Treatments 5–6 control contained, 4 mM ATP, 40 mM glutamate, 5 mM KH₂PO₄, 10 mM KCl, 12 mM MgCl₂, 10 mM MOPS, 10 mM Tris, 0.3 M mannitol, 0.2% bovine serum albumin, mitochondria from glasshouse spinach containing 0.4 mg protein and, where indicated, 2.5 nKat of glutamine synthetase. The final volume (0.60 ml) was incubated at pH 7.8 for 30 min at 22 °C. The reaction was stopped by addition of an equal volume of C₂H₅OH. NH₃ was distilled isothermally at 20 °C into 0.1 ml 1 M H₂SO₄ in an evacuated flask after buffering the mixture at pH 10. The acid solution of (NH₄)₂SO₄ was mixed with 9 ml NH₃-free H₂O, 0.1 ml 2 M NaOH and 0.2 ml 10% (w/v) sodium potassium tartrate and the tubes incubated at 25 °C for 10 min. Nessler's reagent (0.4 ml) (ref. 21) was added and after 30 min at 25 °C absorbance at 435 nm was measured. The Nessler complex was precipitated by adding four pellets NaOH (0.4 g) to each tube, mixing, and storing in the dark for 30 min. The precipitate was washed with 0.5 ml NH₃-free H₂O and suspended in 40 μl for each μmol of NH₃ of 1 M HCl in 10% aqueous C₂H₅OH (ref. 22). After standing overnight at 0–5 °C the insoluble material was removed by centrifugation and 8-μl samples of the supernatant were dried in short lengths of capillary tube at 60 °C. The residues were sealed *in vacuo* (0.2 Pa) in a pyrex tube with appropriate reagents and the ¹⁵N, ¹⁴N content measured by emission spectroscopy²³. Radioactive serine and CO₂ were removed and measured as described previously³.

resulting from the production of ADP from ATP by GS. This was confirmed in oxygen electrode experiments with the same batch of mitochondria.

The above results indicate that reassimilation can occur *in vitro* with GS. To confirm that this also occurs *in vivo* carefully matched flag leaves of wheat (c.v. Sicco) were detached under water, stood vertically with their bases in 3 ml of water and illuminated. Such leaves contained small amounts of NH₃ which increased when they were supplied with ¹⁵N-glycine by the cut

base (Table 2). Measurement of the ¹⁵N content of the NH₃ indicated that the extra amount was derived from glycine. The addition of methionine sulfoximine (MSO) together with the ¹⁵N-glycine increased the amount of NH₃ derived from glycine 4.5-fold. Also, when isolated pea protoplasts were incubated with ¹⁵N-glycine, MSO doubled the production of ¹⁵NH₃. There was, however, a considerable background of ¹⁴NH₃ production by the protoplasts. A similar release of NH₃ from freshly isolated poppy cells has been observed¹⁸. As MSO is a specific inhibitor of GS¹⁹, we conclude that NH₃ produced during the conversion of glycine to serine *in vivo* is normally reassimilated by GS.

On the basis of the above results we propose that photorespiration involves a cycling of nitrogen (see Fig. 1). If the generally accepted estimates of photorespiration and primary assimilation of NH₃ derived from nitrate reduction are correct, then the flux of NH₃ through the photorespiratory nitrogen cycle is an order of magnitude greater than that resulting from primary assimilation. In the scheme, the NH₃ released from glycine is refixed by cytoplasmic GS into glutamine. Glutamine and 2-oxoglutarate move into the chloroplast where, in the presence of reduced ferredoxin and glutamate synthase, two molecules of glutamate are formed; one of these molecules of glutamate becomes the acceptor molecule for another NH₃ molecule and the other provides a transamination equivalent to convert one glyoxylate to glycine while regenerating 2-oxoglutarate (a second transamination equivalent is needed and comes from serine).

As chloroplasts actively assimilate ammonia in the light²⁰, it is possible that chloroplastic GS could be partly involved, but ammonia produced in mitochondria probably first encounters the cytoplasmic GS. The conversion of glutamine to glutamate must occur solely in the chloroplast as that is the only site of glutamate synthase activity in the leaf cell¹⁵. The operation of the cycle would minimise the energy required in the cytoplasm, as the major energy-demanding step is directly coupled to the light-dependent reduction of ferredoxin. Thus, only one ATP of the three generated in the mitochondria during the conversion of two molecules of glycine to serine^{3,4,13} is needed for reassimilation of ammonia. The two molecules of ATP remaining may be used for synthetic reactions in the cytoplasm.

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Table 2 Effect of methionine sulfoximine on NH₃ release from [¹⁵N]-glycine

Solution supplied	Time (min)	Total NH ₃ released (μmol)	NH ₃ released from glycine (μmol)
Wheat leaves			
Water	45	1.7	—
	90	1.4	—
5 mM MSO	45	5.8	—
	90	12.0	—
15 mM ¹⁵ N-glycine	45	2.5	0.6
	90	3.5	1.4
15 mM ¹⁵ N-glycine + 5 mM MSO	45	8.9	2.7
	90	17.4	6.4
Pea leaf protoplasts			
¹⁵ N-glycine + glutamate	60	0.45	0.08
¹⁵ N-glycine + glutamate + MSO	60	0.68	0.16

Wheat flag leaves, each about 0.45 g fresh weight were illuminated for 40 min at 25 °C with the cut bases in water. The water was then replaced by the solutions indicated. The photon flux density at the leaf surface was 800 μEinstein m⁻² s⁻¹ (PAR). Pea leaf protoplasts were isolated by the method of Wallsgrove *et al.*¹⁵ and an amount containing 207 μg chlorophyll incubated at 25 °C in 0.59 ml containing 16.95 mM [¹⁵N]-glycine (99 atom % ¹⁵N), 16.95 mM glutamate, 0.34 M sorbitol, 80 μM MgCl₂, 42 mM Tricine pH 7.5 and, where indicated, 1.93 mM MSO. The reaction mixture was illuminated with a photon flux density of 1,200 μEinstein m⁻² s⁻¹ (PAR) in tubes gassed with air at 300 ml min⁻¹ and shaken at 1 Hz. Wheat leaves were killed in boiling H₂O and homogenised; reaction mixtures containing protoplasts were treated with an equal volume of C₂H₅OH. Measurements of the amount and ¹⁵N content of NH₃ were as in Table 1.

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Bats avoid moving objects more successfully than stationary ones

MICROCHIROPTERANS sense their environment by emitting ultrasonic signals and listening to the echoes¹. The simplest way to measure this ability is by the obstacle-avoidance test^{2–7}, in which a bat flies between an array of stationary wires and the numbers of hits and misses are recorded. We have extended this test and made our bats fly between an array of moving as well as stationary obstacles. We were surprised to find, as reported here, that a bat can avoid moving obstacles more successfully than stationary ones.

Six *Myotis lucifugus* (the little brown bat) with an average maximum wingspan of 23 cm were tested in a room 4.6 m long, 2.2 m wide and 2.1 m high. A wooden frame containing six vertically strung nylon monofilament lines or steel wires (the obstacles) was placed in the centre of the room. The total opening of this barrier (the frame plus the obstacles) through which a bat flew was 1.8 m wide and 1.7 m high. By means of an electric motor the barrier was made to oscillate in the horizontal direction parallel to the alignment of the obstacles, with a maximum amplitude of 9 cm at a rate of 0.39 Hz, which

generated an average speed of 7 cm s⁻¹. Any effect of the movement of the frame itself on the bat's performance was eliminated by surrounding the frame with large wooden panels which were attached to the walls, ceiling and floor. Thus the bat could see the movement of the lines or wires, but not that of the frame. The sound of the moving lines or wires was negligible. All surfaces of the wooden panels, ceiling and walls were covered with convoluted polyurethane foam to reduce the echoes from them. Four different diameters of nylon lines (0.23, 0.37, 0.47 and 0.53 mm) or steel wires (0.32, 0.37, 0.53 and 1.53 mm) were used, one size at a time for each test. The distance between the obstacles was always 30 cm. A bat was made to fly between stationary obstacles at least 32 times before it was made to fly between moving ones another 32 times in order to minimise any effect of spatial memory of the position of the obstacles^{8,9}. Each bat was tested in this way as many times as possible. A hit consisted of collisions of the body or arm with the obstacles. A miss consisted of flights in which the obstacles were not touched or were lightly brushed by a wingtip with no change of flight course.

When an agile bat flew between the stationary obstacles, it negotiated them almost without hesitation. However, when the barrier was moved, the bat hovered first at one side of the flight room before flying between the obstacles. The bat was apparently more cautious when confronted with moving obstacles. Bats tended to fly between the upper central part of the barrier regardless of whether the obstacles were stationary or moving.

Contrary to our prediction, the percentage of misses by all bats was always greater for the moving obstacles than for the stationary ones, regardless of the size of the obstacles. Table 1 summarises the data for four bats which were tested with at least two different diameters of wires or lines. The average percentage of misses obtained in each of the two test conditions differed by 6.5–15%. All these differences are significant (χ^2 test, $P < 0.10$).

Myotis lucifugus flies between an array of obstacles of this sort with an average wingspan of 14 cm (60% of maximum wingspan)^{1,2}. Therefore a model bat with a wingspan of 14 cm was made from wooden sticks, steel wires and tapes. It was thrown at random, in the manner described by Hahn⁶, towards the barrier in order to find out the chance of missing the barrier. Six diameters of nylon line (0.20, 0.27, 0.33, 0.56, 0.83 and 1.0 mm) were used; Table 1 shows only the data obtained with 0.20, 0.56 and 1.0-mm lines. In general, the percentage of misses when the obstacles were moving was not significantly different from that recorded when the obstacles were stationary, regardless of the diameters of the lines. The average percentage of misses for stationary and moving obstacles of six different diameters was 65.2 and 65.4, respectively. These values are 30–40% greater than those reported earlier^{1,2,6}. Because the percentage of misses by bats obtained in either test condition was significantly greater than those found with the model bat, the bats apparently used echolocation to dodge the obstacles.

Table 1 Avoidance of obstacles by bats

Diameters of wires (w) or lines (l) (mm)	Bats tested	Stationary		Moving		Differences in average % misses	χ^2	Significance at 90% correct level $P \leq 0.10$
		Total flights	Average % misses	Total flights	Average % misses			
0.32 (w)	No. 1 & 2	256	72	256	79	7	3.4	Yes
0.37 (w)	No. 1 & 2	352	78.5	320	85	6.5	4.8	Yes
0.53 (w)	No. 1 & 2	256	81.5	220	89	7.5	5.2	Yes
1.53 (w)	No. 1 & 2	192	79.5	192	88	8.5	4.9	Yes
0.23 (l)	No. 3	192	76	192	91	15	16.0	Yes
0.37 (l)	No. 3 & 4	288	80.5	288	91.5	11	14.9	Yes
0.47 (l)	No. 3 & 4	320	80.5	320	90.5	10	13.0	Yes
0.53 (l)	No. 3	128	86	128	93	7	3.4	Yes
0.20 (l)	Model	120	65	120	67.5	2.5	0.2	No
0.56 (l)	Model	128	65	123	70	5	0.7	No
1.0 (l)	Model	323	67	192	64	3	0.4	No

Why was a moving obstacle easier to miss (or perceive) than a stationary one? The answer is probably associated with the fact that carnivorous and insectivorous bats rely on moving prey for survival, and they apparently concentrate more intently on moving objects while hunting. In behavioural experiments, it is essential to present an animal with tasks which are more relevant to situations encountered in its natural environment.

'Motion detectors' have been found in a cat's inferior colliculus¹⁰ and auditory cortex¹¹, and it is likely that the bat's auditory system also contains neurones that are particularly sensitive to an echo source moving in a particular direction and at a particular speed. Indeed, cortical neurones sensitive to a moving sound source have been found in the big brown bat (*Eptesicus fuscus*) by P. Wasserbach (personal communication).

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Pygmy chimpanzee as a possible prototype for the common ancestor of humans, chimpanzees and gorillas

A CONVINCING theory of human origins must clarify man's relationships with living primates and with the ancestral forms known only through fossils. Phylogenetic relationships have previously been determined mainly by anatomical similarities, but now, biochemical similarities provide independent criteria for evolutionary relationships. Albumin and transferrin immunology, immunodiffusion, DNA annealing and amino acid analysis all indicate that chimpanzees, gorillas and humans share a substantial common ancestry, and that the Asiatic apes (gibbons and orangutans) diverged earlier from this lineage¹⁻³. These findings directly conflict with the more widely held view that all the great apes diverged from a common ancestor long after the 'origin' of the evolutionary line leading to modern humans⁴. The molecular data consistently suggest a much more recent origin of the man-chimpanzee-gorilla separation than was previously imagined, namely, in the range of 4-6 M yr ago^{3,5}. These data show that, although the two chimpanzee species (*Pan paniscus* and *P. troglodytes*) are biochemically distinct, they are more closely related to each other than either is to humans or gorillas^{6,7}. The chimpanzees speciated, then, after the initial three-way split. We therefore, here contend that, among living species, the pygmy chimpanzee (*P. paniscus*) offers us the best prototype of the prehuman ancestor. Biochemical, morphological, behavioural and palaeontological data support this proposition and argue for a relatively recent and accelerated divergence of the hominid from the pongid line.

If this cladogram is correct, then an ancestor for the earliest hominids must also be a suitable ancestor for chimpanzees and

gorillas. The morphological similarities between the three groups should logically be derived from the common ancestor, rather than by evolutionary convergence, or by parallelism from distinct primate lineages. In fact, the modern African apes may be viewed as size variants in a single morphotypic series, going stepwise from the smaller *P. paniscus* to the large male gorilla. The scant hominid fossil record during the past 4-8 M yr, and the absence of any fossil chimpanzees or gorillas, forces us to work backwards from the living hominoids to reconstruct a prototype of the common ancestor.

Chimpanzees have generally been considered closer to this prototypic ape than gorillas, because the latter are more specialised morphologically and behaviourally. They are larger and males are twice the size of females; they have a more specialised diet, more restricted habitat and less flexible social behaviour than chimpanzees. Morphologically, pygmy chimpanzees are more 'generalised' in body size, body build and sexual dimorphism than the common species and may provide a better prototype for studying the derivation of the earliest hominids and African apes.

Pygmy chimpanzees on the average weigh less than the average common chimpanzee, but with much overlap: 25-48 kg, compared to 25-60 kg or somewhat more⁸. Pygmy chimpanzees have smaller facial and canine dimensions, smaller average cranial capacities (350 cm³ compared with 390 cm³), and the two species can be discriminated on mandibular length alone⁹.

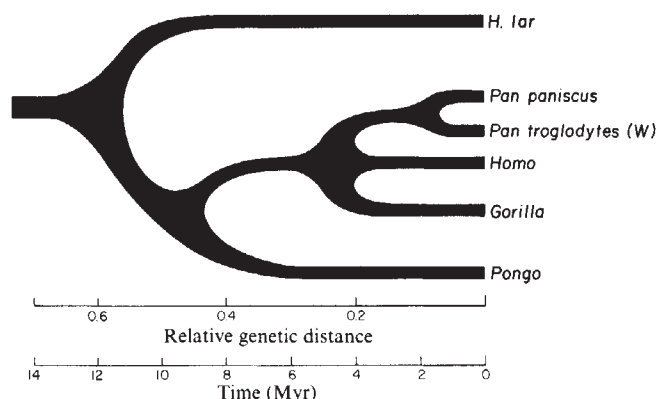


Fig. 1 Molecular cladogram showing genetic relationships of the ape and human lineages.

Pygmy chimpanzees have a narrower trunk and more gracile upper body, as reflected in significantly smaller clavicular length, scapular dimensions and iliac and sacral breadths⁸. Their upper and lower limbs are of about equal length, whereas arms of common chimpanzees are relatively longer.

As to sexual dimorphism, pygmy chimpanzees are moderately sexually dimorphic in body weight, but no dimorphism can be detected in cranial capacity, limb bone lengths or robusticity, or in anterior and posterior dentition¹⁰. They have relatively small and only slightly dimorphic canine teeth¹¹. Overall, this is less dimorphism than occurs in common chimpanzees and no more than occurs in modern humans.

Observations on the behaviour of pygmy chimpanzees, either in their natural habitat in the Zaire River Basin or in captivity, have not been extensive. They feed high in trees, move and feed on the ground^{12,13}, and in captivity walk bipedally more than do common chimpanzees (S. Savage-Rumbaugh, personal communication). They therefore encompass the spectrum of locomotor behaviour of living African hominoids: arboreality, terrestriality and bipedality. Laboratory and field studies reveal pygmy chimpanzees as highly intelligent and flexible in feeding, locomotor, sexual and communicatory behaviour¹⁴⁻¹⁶.

Given these morphological and behavioural data, we maintain that pygmy chimpanzees present a general pattern from

Table 1 Comparison of pygmy chimpanzee and early hominid pelvis, femur and humerus (in mm)

Measurement	Early hominids	Pygmy chimpanzees
Femur length	280*, 280†	293 (264–315)
Femoral head diameter	31†	30.5 (28–34)
Acetabular diameter	37†	36 (32–38)
Innominate length	170†	253 (232–272)
Iliac breadth	113†	97 (80–118)
Sacral breadth	76†	63 (51–70)
Humerus length	235*	285 (250–307)

* AL 288–1 from Hadar, Ethiopia¹⁸.† Sts 14 from Sterkfontein, South Africa. Measurements from A.L.Z. (unpublished data); femur length from Lovejoy²³.

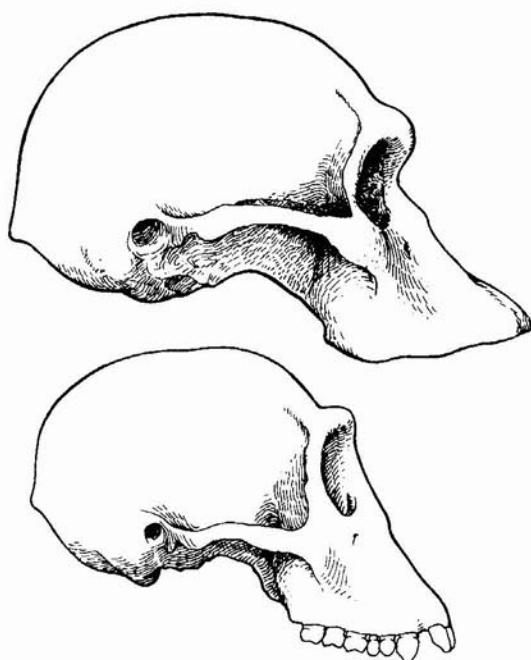
which other African hominoids could have developed. This contention is further supported by comparing pygmy chimpanzees with the earliest hominids.

The fossil record, based on dentition, shows the occurrence of *Australopithecus* by 3.5 M yr ago in the mosaic savannas of Eastern Africa¹⁷. By 3 M yr ago, the hominids were distinct from apes in the broader and shorter pelvis, larger cranial capacity, larger postcanine teeth and somewhat smaller canines^{18,19}.

P. paniscus provides a suitable comparison for *Australopithecus*²⁰; they are similar in body size, postcranial dimensions and, as Figs 2 and 3 show, even in cranial and facial features, although this *Australopithecus* has a larger cranial capacity (485 cm³)²¹ than *P. paniscus* (350 cm³).

Postcranial dimensions are given in Table 1. Femoral length, femoral head and acetabular diameters are very similar. Humerus and innominate lengths, and breadths of sacrum and ilium are different. These findings demonstrate early hominid affinities with apes. Probably the initial acquisition of hominid upright locomotion entailed reduction of upper limb length and broadening of the ilium and sacrum.

Homo and *Pan* (both species) differ in their genomes by approximately 1%, a value characteristic of sibling species²². The genetic changes accounting for the morphological differences between man and chimpanzee may be only a small fraction of that amount. A few regulatory genes may have large somatic effects, whereas the remaining structural genes may be time dependent in their rate of fixation, and of little selective significance.

Fig. 2 Side view, drawn to scale, of Sterkfontein 5 (above) and adult female *P. paniscus* (below).**Fig. 3** Front view, drawn to scale, of Sterkfontein 5 and adult female *P. paniscus*.

The ultimate strength of any hypothesis rests on its compatibility with all lines of evidence, on its ability to withstand falsification and on its power of prediction. We hypothesise (1) that as our knowledge of the fossil record improves, hominids before 3.0 M-yr ago should continue to converge on the pygmy chimpanzee-like condition, cranially and postcranially, as they seem to do dentally, (2) that Pliocene pongids ancestral to chimpanzees and gorillas should do likewise, and (3) that at least one lineage of late Miocene ape (perhaps *Ramapithecus*) should continue to develop evolutionary trends begun in the early Miocene and evolve into a form similar to the extant pygmy chimpanzee.

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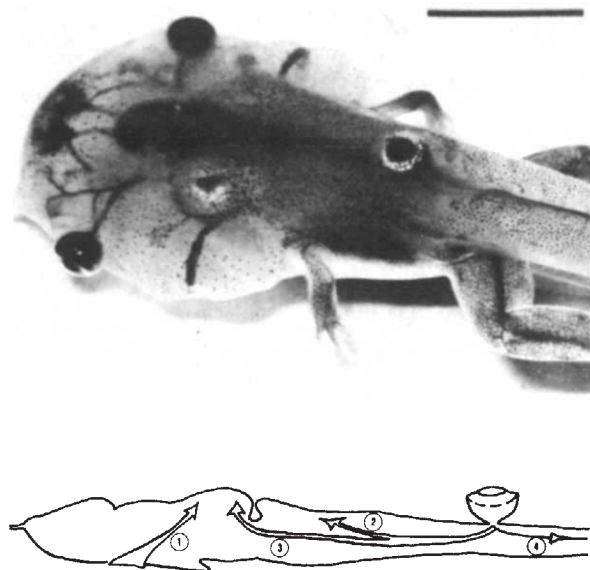
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Axons from eyes grafted in *Xenopus* can grow into the spinal cord and reach the optic tectum

THE transplantation of embryonic eyes in Amphibia is a long established approach for testing the ability of nerve fibres to grow through unusual regions of the central nervous system^{1,2}. As the behaviour of growing ectopic fibres may disclose the mechanisms of axonal guidance^{3–7}, this approach has recently

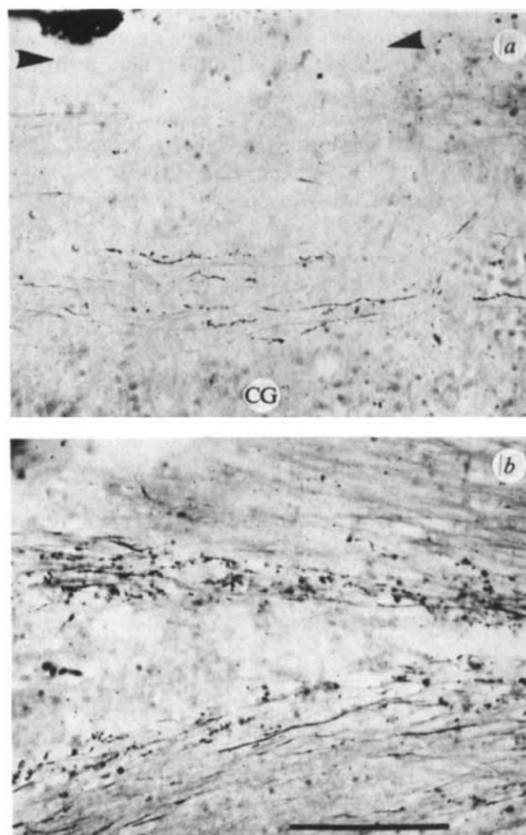
Fig. 1 Tadpole of *Xenopus laevis* with a third eye grafted at embryonic stage 23 (ref. 13) and now (stage 58) growing above the spinal cord. The brain and the normal eyes with their optic nerves can be seen through the transparent tissue of the tadpole head. Embryos were obtained in our laboratory by induced mating¹⁴ of wild animals and were operated on in full-strength Niu and Twitty solution¹⁵ containing antibiotics (10,000 U l⁻¹ of penicillin G and 25 mg l⁻¹ of streptomycin sulphate) and anaesthetic (0.02% MS-222, Sandoz). In the sagittal silhouette of the tadpole CNS the paths of optic fibres issuing from the normal (1) and grafted eyes (2–4) are represented. (1) Normal optic tract terminating in the anterior optic tectum; (2) ectopic fibres terminating in the medulla oblongata in triocular and in blinded (that is, binocularly enucleated) animals; (3) ectopic fibres terminating in the posterior optic tectum in blinded animals; (4) ectopic fibres growing caudally. Scale bar (applies to photograph only), 1 cm.



attracted new interest^{8–12}. We are studying the behaviour of optic fibres entering the spinal cord from a heterotopic eye grafted on to the dorsal trunk region of *Xenopus* embryos^{10,11}. Here we report on the paths used by ectopic fibres to travel in the spinal cord, their possible termination in the hindbrain, and their ability to reach the optic tectum.

Eye vesicles from *Xenopus laevis* embryos at stage 25 (ref. 13) were grafted on to the dorsal region of other embryos at stage 23. Results were studied at larval stages. In 52 tadpoles, the grafts developed into morphologically normal eyes located on the back of the animal, just dorsal to the spinal cord and at different distances from the brain (Fig. 1). Twenty animals (out of the 52 mentioned above) were cut sagittally and stained by Holmes' silver method¹⁶ for histological analysis: seven of the grafted eyes had developed an optic nerve whose fibres entered the spinal cord. These fibres seemed to grow mainly rostrally. We have confirmed this by tracing the paths of degenerating fibres in the remaining 32 tadpoles. Twelve of these animals had a considerable number of degenerating fibres in the spinal cord (Fig. 2). In each case we found most argyrophilic debris rostral to the graft; a smaller amount occurred caudally. Quantification of fibres in this type of preparation is not possible, but we estimate that approximately three-quarters of the fibres grew rostrally. No specific path seems to have been used by these optic axons; degenerating fibres were broadly distributed in the dorsal half of the white matter of the spinal cord (Fig. 2a). In particular, they

Fig. 2 Degenerating fibres in the spinal cord of experimental tadpoles. Horizontal section; rostral is left. The grafted eye was opened and the retina was gently peeled out. After 24 h the animals were fixed in neutralised 10% formalin and processed to demonstrate degenerating nerve fibres and terminals by a modification of Nauta's reduced silver method¹⁷. a, The ectopic fibres are localised near the central grey (CG); they were found bilaterally in various positions inside the dorsal white matter, but never close to the pial surface (between arrows). b, Degenerating fibres in the dorsal funiculi; in this region (hindbrain), they are diverging. Scale bar, 100 µm.



were not grouped near the dorso-lateral surface of the spinal cord, as described by others^{9,12}. In two cases a large proportion of degenerating fibres was concentrated in the dorsal funiculus (Fig. 2*b*). In all cases they occurred bilaterally.

The possible termination of ectopic fibres was studied in the same set of 32 animals mentioned above. In an initial series of 17

12 h after eye transplantation and 15 h before outgrowth of optic fibres¹³. We processed the tadpoles (stages 52–57) to trace the ectopic fibres. In this second series (the remaining 15 tadpoles of the 32 mentioned above), we found three cases with extensive terminal degeneration in the optic tectum, as well as two cases with a weaker tectal projection from the ectopic eye. Terminal degeneration was bilateral and diffuse in the superficial layer of the posterior part of the optic tectum (Fig. 4*a*) and, ventrally, near the nucleus isthmi. In these five cases, bilateral terminal degeneration was also found in the solitary tract.

Sharma⁸ and Constantine-Paton and Capranica⁹ have grafted eyes in *Rana* on to the dorsal diencephalon and the internal ear region, respectively. Their results led to the suggestion⁹ that optic fibres are inherently specified for a dorso-caudal growth, relative to the overall polarity of the brain. The extensive growth of ectopic fibres in a rostral direction along the spinal cord, previously reported by us¹¹ and by Katz and Lasek¹², does not agree with this suggestion. The varied location of degenerating fibres along the spinal cord described here does not support the idea that axons from grafted eyes are growing 'in a specific set of

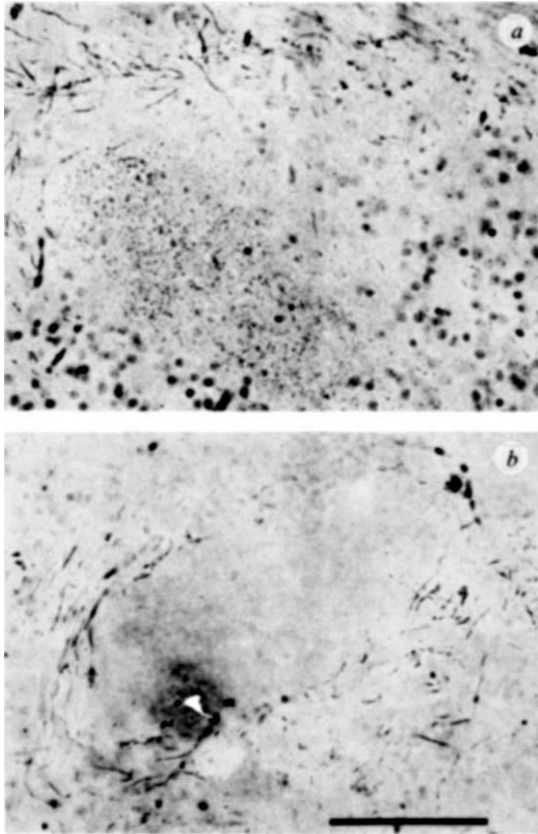
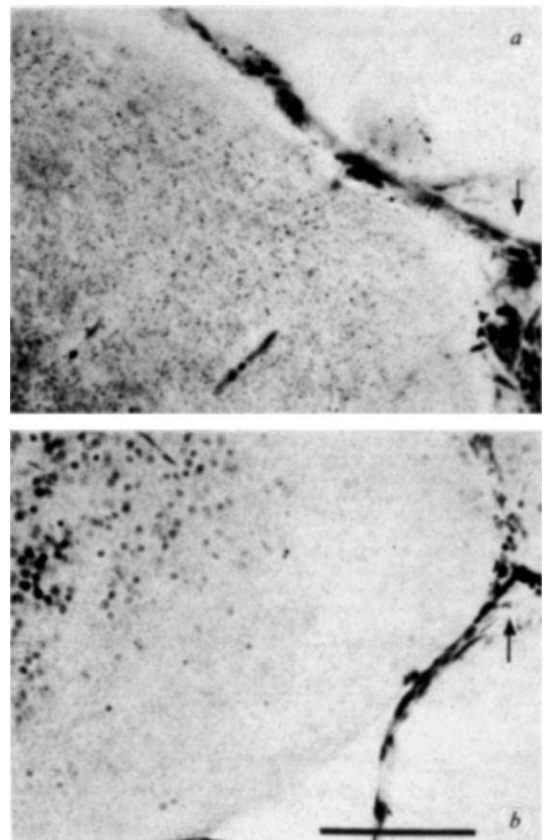


Fig. 3 *a*, Same experimental situation as Fig. 2. Horizontal section; rostral is left. Terminal degeneration is inside the right solitary tract (the phenomenon was bilateral). The nucleus of the solitary tract is not clearly delimited in Amphibia; neurones surrounding the solitary tract send their dendrites into it to receive the terminals from the tract¹⁸. Lateral to the solitary tract (top right) are some degenerating fibres. *b*, Left solitary tract of a control animal in which the dorsal spinal cord was sectioned 24 h before death. No terminal degeneration is present. Scale bar, 100 μ m.

tadpoles with a third eye grown on their back, we found four animals (stages 52–57) with evidence of terminal degeneration in the hindbrain (in three tadpoles there was no terminal degeneration in spite of the presence of optic fibres in the spinal cord). The terminal degeneration was restricted to the 'nucleus' of the solitary tract (Fig. 3*a*)—always occurring bilaterally and along its entire extent—with the exception of one case showing weak terminal degeneration lateral to the nucleus isthmi. To establish whether the above results were due to damage of the spinal cord during enucleation, control experiments were carried out in four normal tadpoles by transecting the dorsal half of the spinal cord and adopting a 24-h survival time, as for grafted eyes. No terminal degeneration was found in the solitary tract (Fig. 3*b*).

To understand why ectopic fibres did not reach the optic tectum (the main natural target of optic fibres¹⁹), we removed both normal eyes of the host embryos at stage 26, that is, about

Fig. 4 *a*, Same experimental situation as Fig. 2, except that both normal optic vesicles of the host embryo were removed 12 h after eye transplantation. Horizontal section; rostral is left. Terminal degeneration is in the posterior part of the right optic tectum (the phenomenon was bilateral). During larval stages the posterior optic tectum is not yet laminated. On the extreme right is the postero-lateral corner of the tectum and its border (arrow) with the cerebellum. In grafted and blinded tadpoles the terminal degeneration in the tectum was always accompanied by terminal degeneration in the solitary tract (see Fig. 3*a*) and by degenerating fibres in the spinal cord (see Fig. 2). *b*, Corresponding region of the left optic tectum of a triocular tadpole whose grafted eye successfully innervated the spinal cord. No terminal degeneration is present. The specimen shown here is the same one whose solitary tract is represented in Fig. 3*a*. Scale bar, 100 μ m.



pathways^{11,12}. The adoption of a certain route of growth and the choice of the direction of growth do not necessarily depend on the same mechanism. Are directional cues encoded in the overall polarity of the brain or in the location of the target relative to the graft? For optic fibres growing rostrally in the spinal cord, the latter would seem to be the case. The mechanism by which a target organ could guide axonal growth remains to be explained.

The plasticity of growing optic fibres is proved not only by their ability to follow unusual pathways in the CNS, but also by their anomalous termination. We found that rostrally growing ectopic fibres terminate in the nucleus of the solitary tract. Future ultrastructural and electrophysiological investigation will show whether functional synapses are formed within this nucleus, which normally receives visceral signals. Ectopic fibres were found to terminate in the caudal part of the optic tectum only in blinded animals. It is difficult to explain the lack of such projection in triocular animals in terms of competition for terminal space; at very young larval stages (when ectopic fibres could have reached the tectum) normal optic fibres terminate in the rostral part of the tectum, which becomes fully colonised only after stage 57 (refs 20, 21).

It will be necessary to analyse in detail the embryological nervous system as it interacts with growing ectopic axons, before attempting to understand the mechanisms underlying such interaction. In our view the adoption by the ectopic axons of a certain route may depend on the options offered by the experiment (for example, level of penetration into the CNS), and on a hierarchy of preferred interactions with the surrounding tissues (through cell surface recognition²²). The present work focuses attention on the role of a target, in this case the optic tectum; both its position (relative to the graft) and its input (from normal eyes) seem to affect the behaviour of optic fibres issuing from the grafted eye.

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Microscopic epididymides in testicular feminisation

MICE bearing the X-linked testicular feminisation (*Tfm*)¹ gene in the hemizygous (X/Y) state have been presumed to have no traces of the male genital system. Unlike normal genetic males², they are apparently unresponsive to the androgens produced by the developing testes^{3,4}, and it has been presumed that derivatives of the embryonic mesonephric (Wolffian) duct system (MDS) are unable to survive in *Tfm*/Y males. I describe here, however, microscopic components of the complex comprising rete testis, efferent ductules, epididymis and, sometimes, vas deferens in adult *Tfm*/Y mice. These structures are unrelated to the effects of the presumed mutation *O^h*, to which the microscopic epididymides of some *Tfm*/Y mice have been ascribed⁵.

Because it has not been known whether *Tfm*/Y mice without *O^h* possess microscopic epididymides, I examined such adults (6-33 weeks). As the *O^h* X chromosome carries the blotchy (*Blo*) mutation, mice with *Blo* (*n* = 3) and without (*n* = 2) were studied. They were bred from stock obtained from U. Drews, Münster, and J. Tønnes Nielsen, Aarhus, respectively. Both strains originate from Harwell.

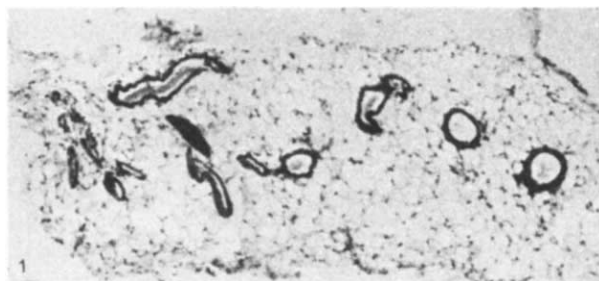
Right and left gonads, with paragonadal material from the caudal pole of the kidney to the internal inguinal ring, were fixed and embedded *en bloc* in paraplast. Serial 7-12 µm sections of the entire block (~1,500 sections per block) were stained with haematoxylin and eosin, and examined by light microscopy.

All the mice possessed rete testis, efferent ductules and microscopic epididymides on both sides (Fig. 1), and in each case there was continuity between epididymis and rete. The epididymides resembled those reported in *Tfm O^h Blo*/Y mice⁵. A vas deferens could sometimes be identified. The epithelium lining these mesonephric derivatives was mainly of the low cuboidal type attributed to *Tfm* cells⁵⁻⁸ in *Tfm*/+ heterozygotes masculinised by the sex-reversal gene *Sxr*⁹. However, isolated areas of taller, stereociliated or ciliated epithelium were seen (Fig. 2). No simple correlation between age and the degree of development of MDS was evident.

Microscopic epididymides are thus evidently a trait of *Tfm*, not of the *O^h* state. My observations also have a bearing on previous conclusions drawn from data on masculinised *Tfm*/+ heterozygotes. The assumption that the cuboidal epithelial cells in these heterozygotes represent surviving *Tfm* cells receives some support. There is, however, no need to suggest inter-cellular diffusion or metabolic co-operation⁵⁻⁸; these cells are clearly capable of independent survival. Furthermore, stereocilia or cilia can no longer be used to distinguish *Tfm* from wild type cells⁸ in such heterozygotes. Indeed, there is no reliable criterion for this purpose.

The survival of epididymal and related structures in adult *Tfm*/Y mice suggests that the cells involved are either not dependent on testosterone or they are not entirely insensitive to it. Because the enzyme β-glucuronidase is histochemically

Fig. 1 Cross-section of the epididymis of a 9-week-old *Tfm*/Y mouse without *Blo* (×60).



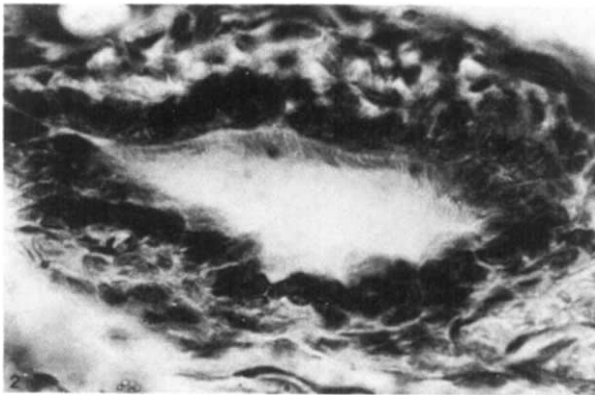


Fig. 2 Tubule cross-section of epididymis of a 9-week-old *Tfm/Y* mouse without *Blo* ($\times 750$), showing ciliated and/or stereociliated epithelium. (Both cilia and stereocilia appear to be present in normal mouse epididymides¹³.)

inducible by androgen in normal epididymis¹⁰, as in the kidney¹¹, studies of that enzyme in the microscopical epididymides may resolve this problem.

Not only the MDS but also the testis itself is abnormally small in *Tfm/Y* hemizygotes. It is possible that initial embryonic determination, establishment and maintenance of both testis and MDS are independent of testosterone but that normal maturation of both is under its control. Thus in one possible interpretation of my data, *Tfm* cells in these organs may be insensitive to androgens, but survive because only some aspects of normal function and development are dependent on such hormones. Alternatively, ontogenetic changes in affinity of repressor molecules for inducer (androgen), or for receptor sites, or developmental loss of the latter, could account for my observations.

Rete components of the MDS are essential for initiation of meiosis in the mouse testis¹², and in *Tfm/Y* mice meiosis is indeed initiated and proceeds to metaphase¹. On this basis the presence of a functional rete in *Tfm* mice could have been predicted. Conversely, it can be deduced from my observations that at least part of an MDS composed entirely of *Tfm* cells is functionally competent, independent of any metabolic co-operation with wild type cells. This system may be of value in studies of developmental aspects of hormonal competence.

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Uniparental inheritance is promoted by delayed division of the zygote in *Chlamydomonas*

SEXUAL REPRODUCTION in *Chlamydomonas reinhardtii*, a heterothallic, unicellular green alga, yields two types of zygotes: the thick-walled, dormant zygospor (meiotic), and less frequently, the vegetative zygote which divides mitotically soon after initial gamete fusion to produce stable diploid progeny^{1,2}. Although vegetative zygotes account for less than 5% of the products of gamete fusion, their diploid progeny can be easily

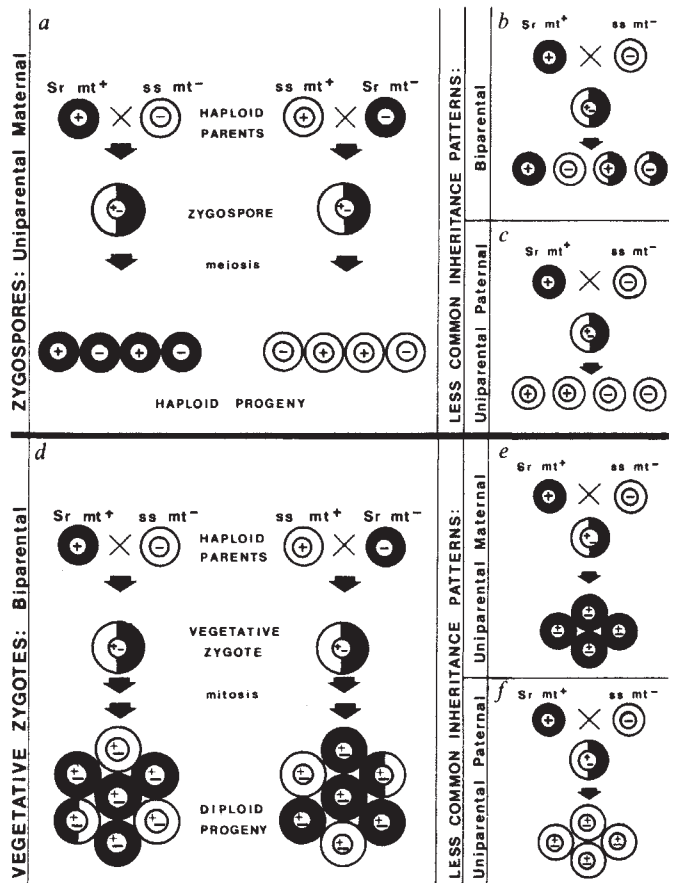


Fig. 1 Comparison of chloroplast gene transmission patterns in zygospor and vegetative zygotes of *C. reinhardtii*. Chloroplast gene transmission in zygospor: **a**, the chloroplast streptomycin-resistance mutation, *Sr* (shaded circles) is transmitted to all four meiotic products by greater than 90% of the zygospor if introduced through the *mt*⁺ parent, and is transmitted to none of the progeny if introduced through the *mt*⁻ parent. **b**, Biparental inheritance, observed for less than 10% of the zygospor, results in the transmission of both the *Sr* and wild-type *ss* (open circles) chloroplast alleles to the meiotic products. **c**, Paternal zygospor, transmitting only the chloroplast alleles of the *mt*⁻ parent, represent less than 1% of the zygospor in most crosses. Regardless of the pattern of chloroplast gene transmission, the nuclear mating-type alleles (indicated by + and -) segregate 2:2 among the meiotic products. Chloroplast gene transmission in vegetative zygotes: **d**, Biparental vegetative zygotes, transmitting the chloroplast alleles of both parents to their diploid progeny account for greater than 70% of the vegetative zygote population in most crosses. Segregation of parental chloroplast alleles occurs gradually and is completed within 20 generations. **e-f**, less than 25% of the vegetative zygotes exhibit uniparental chloroplast gene transmission, with uniparental maternal inheritance (**e**) usually being much more common than uniparental paternal (**f**). (The mendelian auxotrophic markers required for the selection and identification of vegetative zygotes² are not indicated in the figure.)

Table 1 Chloroplast gene inheritance patterns in zygospores and vegetative zygotes of *C. reinhardtii*

Cross	Chloroplast phenotypes	Chloroplast inheritance patterns (frequency)					
		Zygospores			Vegetative zygotes		
		BP	UPm	UPp	BP	UPm	UPp
<i>a</i> <i>Nr ss mt</i> ⁺ × <i>ns Sr mt</i> ⁻ <i>ns Sr mt</i> ⁺ × <i>Nr ss mt</i> ⁻	Neamine-resistant × Streptomycin-resistant Streptomycin-resistant × Neamine-resistant	0.02 0.01	0.97 0.97	0.01 0.02	0.90 0.94	0.08 0.06	0.02 0
		Chloroplast inheritance patterns (frequency)					
		Isolated under continuous light			Vegetative zygotes		
		BP	UPm	UPp	BP	UPm	UPp
<i>b</i> <i>Nr ss mt</i> ⁺ × <i>ns Sr mt</i> ⁻ <i>Er sps mt</i> ⁺ × <i>es Spr mt</i> ⁻ <i>es Spr mt</i> ⁺ × <i>Er sps mt</i> ⁻	Neamine-resistant × Streptomycin-resistant Erythromycin-resistant × Spectinomycin-resistant Spectinomycin-resistant × Erythromycin-resistant	0.86 0.95 0.66	0.14 0.02 0.34	0 0.03 0	0.32 0.57 0.18	0.63 0.25 0.68	0.05 0.19 0.14

Opposite mating types in all of the above crosses also carried recessive complementary mendelian mutations conferring a requirement for exogenous arginine. Thus, vegetative zygotes were selected by plating mated cells on medium lacking arginine (acetate was provided as an organic carbon source for all isolations). Zygospores were matured in darkness for 7–10 d on the same medium; germination was subsequently induced by transfer to medium containing arginine and incubation in the light. 50–64 vegetative zygotes and/or 100–200 zygospores were analysed for transmission of the chloroplast antibiotic resistance markers. Chloroplast inheritance patterns are abbreviated as follows: BP, biparental; UPm, uniparental maternal; UPp, uniparental paternal.

selected on minimal medium by crossing haploid strains which carry complementing auxotrophic mendelian mutations². I report here that differences in chloroplast inheritance observed between meiotic and mitotic zygotes of *Chlamydomonas* are correlated to the timing, rather than the nature, of their first divisions. Environmental conditions which delay the first post-mating division of vegetative zygotes result in uniparental inheritance while favourable post-mating environments promote biparental inheritance of chloroplast genes.

Chloroplast inheritance in *C. reinhardtii* is generally described as uniparental since 90% or more of the zygospores transmit to their progeny only those chloroplast alleles derived from the 'maternal' (mating-type plus, *mt*⁺) parent^{3–5}. Exceptional zygospores may transmit the chloroplast alleles of both parents (biparental zygospores), or rarely, may transmit only those alleles initially carried by the 'paternal' (mating-type minus, *mt*⁻) parent (Table 1a, Fig. 1). Nearly all investigations on chloroplast inheritance in *Chlamydomonas* have centred on the meiotic zygospore. The notable exceptions are studies by Gillham^{6,7} reporting a tendency toward biparental chloroplast gene transmission in vegetative zygotes (see Fig. 1) and suggesting a correlation between their inability to undergo meiosis and their failure to exclude paternal chloroplast alleles.

In work to be reported elsewhere, I found that the culture medium used to isolate vegetative zygotes affected the pattern of chloroplast gene transmission. Media promoting the most rapid vegetative growth also promoted biparental inheritance. This suggested that the observed differences in inheritance patterns between zygospores and vegetative zygotes (see Table 1a, Fig. 1) might result from differences in the timing, rather than in the nature (meiotic or mitotic), of their first post-mating divisions. To test this hypothesis I attempted to induce a temporary dormancy in the vegetative zygotes. Experimental procedures which can be used to promote maturation and extend dormancy in zygospores include nitrogen starvation⁸ and incubation in darkness⁹; it seemed likely that either of these treatments might also delay the first division of vegetative zygotes.

When the vegetative zygotes were selected in darkness, the recovery of macroscopically visible zygote clones was delayed as

compared to control isolations under continuous illumination. Furthermore, chloroplast gene transmission shifted toward uniparental maternal inheritance (Table 1b). By returning the vegetative zygotes to the light after various periods in darkness, a stepwise change in the inheritance pattern can be observed (Fig. 2a). However, as vegetative zygotes (but not zygospores) do eventually divide in darkness (if an organic carbon source is provided), this method does not make it possible to delay the first division indefinitely.

In contrast, the nitrogen-free medium used to induce gametogenesis and mating in *C. reinhardtii*¹⁰ also blocks post-mating cell division. By continuing to starve cells for nitrogen after mating, division of the vegetative zygotes can be delayed for at least 6 d with no subsequent loss in viability. Increasing the duration of the post-mating nitrogen starvation period led to a decrease in the frequency of biparental chloroplast gene transmission until uniparental maternal inheritance, normally associated with zygospores, became the predominant inheritance pattern in the vegetative zygote population also (Fig. 2b).

To verify that the experimental conditions of dark incubation or nitrogen starvation delayed the first division of the vegetative zygotes, and to estimate the approximate extent of this delay, subsamples of the mating population were plated on standard agar-solidified medium and incubated in various conditions. Periodically, sterile liquid was added to the surface of the sample plates and the adherent cells were respread. Respreading after zygote division had occurred would be expected to result in an increase in the number of colonies subsequently recovered.

In this manner, it was shown that: (1) incubation in darkness delayed the onset of the first division by approximately 40 h (Fig. 3a); (2) no division of the vegetative zygotes occurred during maintenance in nitrogen-free medium (Fig. 3b), and (3) under continuous illumination, division always occurred within 30 h after the return of available nitrogen, regardless of the duration of the previous dormant (nitrogen-starvation) period.

While these observations demonstrate a previously unrecognised potential for uniparental inheritance in vegetative zygotes, they perhaps more importantly reveal a remarkable influence of external environment on chloroplast inheritance. This influence,

which extends also to zygospores of *C. reinhardtii*^{8,11,12}, must be borne in mind when defining laboratory conditions for studies on chloroplast inheritance, and when interpreting and comparing the results of such experiments conducted in different laboratories.

The correlation between delayed division and uniparental inheritance suggests an alternative to previous arguments that uniparental inheritance is, in itself, of direct selective advantage^{13,14}. The molecular mechanism responsible for uniparental inheritance in *Chlamydomonas* apparently involves extensive degradation of zygotic chloroplast DNA (normally present as multiple copies of a small genome in vegetative cells)¹⁵⁻¹⁸. Perhaps chloroplast genome polyploidy is a luxury permitted only in an optimum environment, and reduction in ploidy level represents an energy-conserving measure essential to the maintenance of viability in a hostile environment. Viewed in this way, uniparental inheritance is seen as an indirect consequence of direct selection, in evolutionary history, for the dormant, highly resistant stage (the zygospore) in the life cycle of *Chlamydomonas*.

Fig. 2 Effect of post-mating environmental conditions on the transmission of chloroplast genes in vegetative zygotes of *C. reinhardtii*. *a*, Effect of post-mating incubation in darkness. Chloroplast mutants resistant to neamine (*mr*⁺ parent) and to streptomycin (*mr*⁻ parent) were crossed following standard procedures^{8,9} and the mated cells plated on acetate-supplemented minimal medium for the selection of vegetative zygotes (complementary recessive nuclear mutations to arginine auxotrophy were carried by opposite mating-types). Subsamples of the selection plates were incubated in darkness for the periods indicated and were then transferred to continuous light. Resultant zygote colonies were replica-plated to medium supplemented with 75 $\mu\text{g ml}^{-1}$ neamine and to medium supplemented with 100 $\mu\text{g ml}^{-1}$ streptomycin. Biparental vegetative zygote clones are defined as those which yield growth on both of the antibiotic test media. *b*, Effect of post-mating nitrogen starvation. Parental strains are as described in (*a*) above. Gametogenesis and mating were carried out in nitrogen-free medium and the mated culture held in the same medium for 6 d. Samples were periodically withdrawn at the times indicated and plated on nitrogen-containing, acetate-supplemented standard medium under continuous illumination. Biparental zygote clones were defined as described above. Open and shaded circles represent separate repetitions of the same cross. 64 vegetative zygote clones were analysed from each dark incubation (*a*) or nitrogen-starvation (*b*) period. Vertical bars indicate 95% confidence limits.

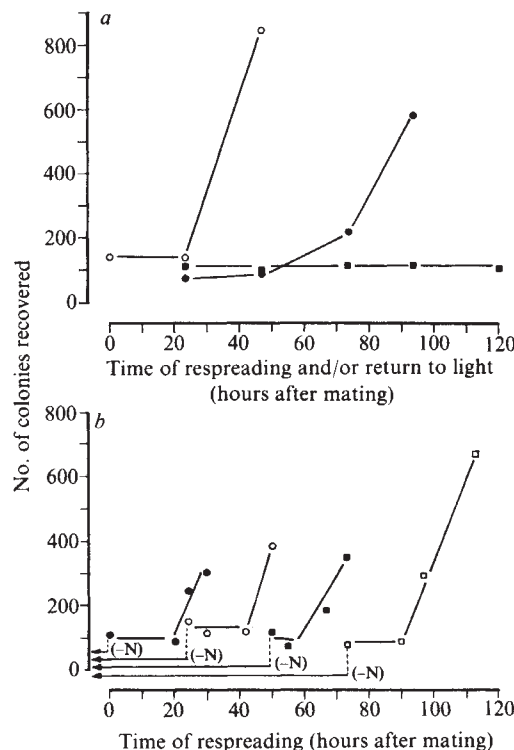
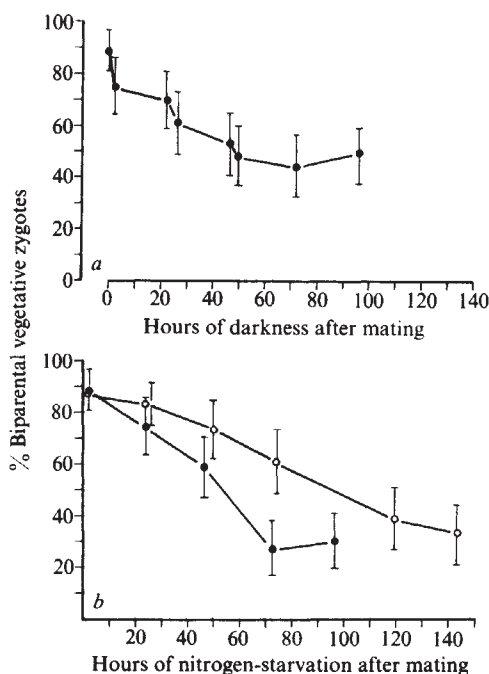


Fig. 3 Effect of post-mating environmental conditions on the timing of the first division of vegetative zygotes of *C. reinhardtii*. *a*, Effect of post-mating incubation in darkness. Mated cells were plated on acetate-supplemented minimal medium and incubated either under continuous illumination ($\circ$) or in darkness ($\bullet$, $\blacksquare$). At the times indicated, the adherent cells were respread. Sample plates incubated in darkness were returned to the light at each indicated time both with ($\bullet$) and without ($\blacksquare$) respreeding. The time of the first vegetative zygote division can be estimated by comparing the number of colonies recovered on respread and non-respread plates. *b*, Effect of post-mating nitrogen starvation. Mated cells were removed from nitrogen-free medium and were plated on acetate-supplemented minimal medium within 2 h after mating ($\bullet$), and after 24 ($\circ$), 48 ($\blacksquare$), or 72 ($\square$) h of post-mating nitrogen starvation. Adherent cells were respread periodically after plating (the first symbol, in each case, indicates the average colony count on non-respread plates).

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Sensitivity of corticosteroid-dependent insulin-resistant lipogenesis in marrow preadipocytes of obese-diabetic (db/db) mice

It is well known that adipocytes with specific cell-membrane insulin receptors respond to insulin by increasing glucose uptake, oxidation and lipogenesis in the presence of glucose¹⁻⁴. Maintenance of adipocyte insulin sensitivity during a state of relative muscle cell insulin resistance has been considered a possible mechanism for obesity in diabetes mellitus⁵. I now report that a distinct cell phenotype resists insulin but accumulates lipid in response to corticosteroid. Furthermore, corticosteroid-induced lipogenesis is significantly greater in marrow preadipocytes of mutant diabetic-obese mice.

Weanling 6-8 week-old NIH/Swiss, BALB/c (National Cancer Institute), C57 BL/KsJ-db (genotype *db+/db+*) diabetic mice and their control littermates *db+/m+* and *m+/m+* (Jackson Laboratories) were killed by cervical dislocation and the marrow contents of a single femur and tibia were flushed in 10.0-ml volumes of Fisher's medium (Gibco), supplemented with 25% serum (donor horse, fetal calf or goat), into plastic flasks (Corning) as before⁶ and standardised to 2.0×10^7 cells per culture. A sample of donor horse serum (Flow Laboratories, Manchester) which has been reported to induce lipid accumulation in marrow cells⁶, was provided by Dr T. M. Dexter. The serum factor(s) stimulating lipid accumulation by these cells and their role in maintaining haemopoiesis *in vitro* are unknown.

Fig. 1 Morphological appearance of the adherent cell population in a 4-week marrow culture grown in: *a*, 'deficient' lot 4107064 horse serum ($\times 100$); or *b*, the same serum supplemented with 10^{-7} M hydrocortisone sodium hemisuccinate ($\times 200$).

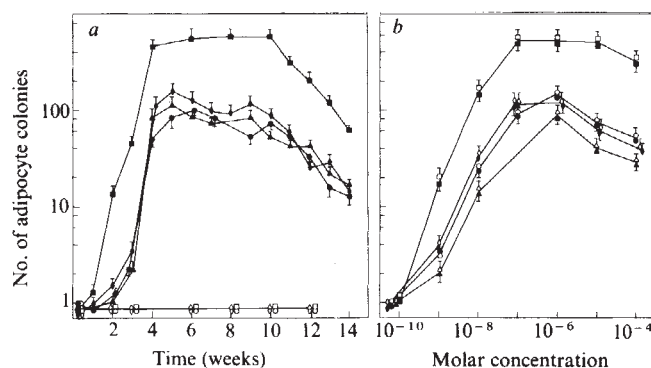
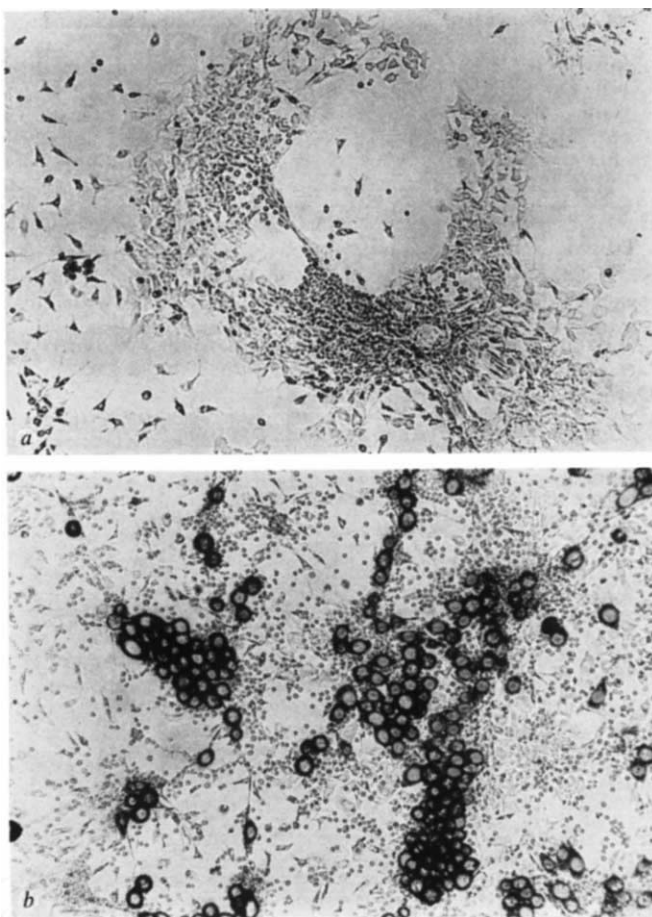


Fig. 2 Kinetics and sensitivity of corticosteroid-induced adipocyte colony formation in obese-diabetic compared with control mouse marrow. *a*, Results are the mean of at least three cultures at each time grown in 10^{-7} M insulin (open symbols) or 10^{-7} M hydrocortisone sodium hemisuccinate (closed symbols) for each mouse strain; *b*, results are the mean of total adipocyte colonies per flask scored at week 6 for at least three cultures at each concentration of hydrocortisone sodium hemisuccinate alone (open symbols) or in the presence of 10^{-7} M insulin in addition to steroid (closed symbols). $\square$, C57BL/Ks-J (db/db); $\circ$, C57BL/Ks-J (db/m or m/m); $\diamond$, NIH/Swiss; and $\triangle$, Balb/c.

Cultures were incubated at 33, 31 or 37 °C and were depopulated and fed by weekly removal of half the flask volume (5.0 ml) and replacement by an equal volume of fresh medium. Cultures were recharged at week 4 by addition of the contents of a second mouse femur⁶. Sera from several sources were supplemented with each of several concentrations of hormones listed in Table 1, and cultures were scored daily for the number of macroscopic adipose cell-containing colonies per flask and of adipose cells per high-powered microscopic field (Olympus inverted microscope). As Table 1 shows, Manchester serum supported formation of more than 100 macroscopic adipose cell colonies per flask by week 4. This was associated with more than 100 adipocytes per high-powered field in the centre of such a colony. Each of 16 lots of horse serum (Flow Laboratories, Rockville), eight lots of fetal calf serum (Colorado Serum Co. or Gibco), or goat serum in concentrations ranging from 10 to 50% failed to stimulate adipose cell differentiation (Table 1). Addition of arachidonic acid (Nu-chek, Elysian, 0.5-500 $\mu\text{g ml}^{-1}$), bovine insulin (Sigma, 0.5-500 $\mu\text{g ml}^{-1}$), butyric acid, dimethylsulphoxide or idodeoxyuridine to deficient sera failed to stimulate adipocyte differentiation.

In contrast, addition of hydrocortisone sodium hemisuccinate (Solu-Cortef, Upjohn) in doses between 0.05 and 500 ng ml^{-1} (10^{-10} - 10^{-6} M) resulted in dose-dependent adipose cell differentiation (Fig. 1) with 12 of the 16 lots of donor horse serum and to a lesser extent with fetal calf or goat serum (Table 1 and Fig. 2). Addition of corticosteroid to Manchester serum did not further increase the numbers or size of adipocyte colonies. Removal of corticosteroid from the medium after adipocytes had differentiated was associated with gradual loss of adipocytes. Thus, the continued presence of corticosteroid was absolutely required.

Other corticosteroids were effective as well as the mineralocorticoid, *d*-aldosterone and the 17-hydroxy adrenal corticosteroid corticosterone (Table 1). Oestrogenic, androgenic or progestational steroids were inactive in the range 10^{-10} - 10^{-4} M (Table 1). With optimal corticosteroid concentration, 10^{-7} M, adipocyte differentiation increased directly with serum concentration in the range 0.5-25.0%, thus arguing against the presence of a serum inhibitor. Corticosteroid-induced adipocyte differentiation was observed in cultures at 31 and 37 °C but was optimal at 33 °C.

Marrow from C57BL/Ks-J (db/db), (db/m), (m/m), BALB/c and NIH/Swiss mice was next compared for steroid sensitivity of adipocyte differentiation. There was no detectable lipid accu-

mulation in *db+db+* or control littermate marrow preadipocytes in response to increased fatty acid concentration or insulin (Fig. 2a). When 2×10^7 marrow cells were cultured in 10^7 M hydrocortisone, *db/db* preadipocytes yielded significantly more adipocyte colonies after 8 weeks than did preadipocytes from control littermates. Figure 2b shows that this reflected a true increase in sensitivity of *db/db* cells to corticosteroid. Significant adipocyte differentiation was detected at a corticosteroid concentration (10^{-9} M) 100 times lower than that required by marrow from control littermates.

Evidence that marrow preadipocyte differentiation was truly insulin independent was provided as follows. Cultures were preincubated in insulin (10^{-10} – 10^{-7} M) for up to 4 weeks with no alteration in the number of adipocyte colonies induced after the addition of 10^{-7} M corticosteroid at week 4. Addition of insulin to corticosteroid-containing cultures of *db/db* or control marrow did not further stimulate or block lipogenesis (Fig. 2b).

When adipocytes differentiated in hydrocortisone-treated cultures 5–7 times as many adherent cells and 100–500 times as many nonadherent cells were detected in culture flasks at weeks 4, 8, 12 and 14 as in untreated cultures. There was persistent proliferation of pluripotent haemopoietic stem cells (CFUs) and GM-progenitor cells (CFUc) for at least 14 weeks in steroid-treated cultures while untreated cultures failed to support haemopoiesis after week 6 (Fig. 3). It is not known whether the increased numbers of CFUc and CFUs arose from corticosteroid-induced cells or were present in the inoculum and required corticosteroid for their proliferation or maintenance of a growth stimulatory microenvironment. Recent data indicate that when preadipocyte differentiation is blocked by Kirsten murine sarcoma virus, *in vitro* haemopoiesis¹⁶ is inhibited.

Epididymal adipocytes resist lipogenesis by insulin after insulin-preincubation and this has been shown to represent

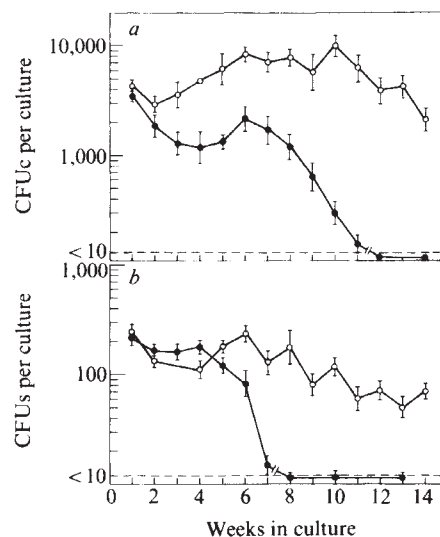


Fig. 3 Influence of corticosteroid-induced marrow preadipocyte differentiation on pluripotent haemopoietic stem cell and GM-progenitor cell proliferation *in vitro*. Cultures of NIH/Swiss mouse marrow were collected weekly by removal of half the flask volume (5.0 ml) and assayed for total numbers of spleen colonies (CFUs) and *in vitro* GM colonies (CFUc)⁽⁶⁾ detected per 10^5 cells. Results are corrected for the total number of colony-forming cells in the 5.0-ml removed and are the mean and standard error for at least three flasks at each week. *a*, CFUc for 25% 'deficient' lot 4107064 horse serum alone, ●; or with 10^{-7} M hydrocortisone sodium hemisuccinate, ○. *b*, CFUs for 25% 'deficient' horse serum alone, ●; or with 10^{-7} M hydrocortisone sodium hemisuccinate, ○.

Table 1 Effect of steroids on *in vitro* mouse bone marrow preadipocyte differentiation

Serum with or without steroid (molar concentration)	No. adipose cell islands/culture					No. adipocytes/ high powered field				
	Weeks in <i>in vitro</i> culture									
	1	2	4	8	15	1	2	4	8	15
Fetal calf serum										
Colorado alone	0	0	0	0	0	8	3	1	0	0
Colorado+hydrocortisone										
Sodium hemisuccinate (10^{-7})	10	16	45	30	17	50	75	85	75	71
Gibco+hydrocortisone										
Sodium hemisuccinate (10^{-7})	8	35	18	21	16	13	57	70	65	60
Gibco alone	0	0	0	0	0	4	0	0	0	0
Goat serum alone	0	0	0	0	0	8	0	0	0	0
Goat serum+hydrocortisone										
Sodium hemisuccinate (10^{-7})	8	10	6	1	1	4	18	25	25	18
Horse serum										
Flow Laboratories (Manchester)	33	40	>100	>100	53	79	>100	>100	>100	50
Flow Laboratories (Rockville)										
Lot 4107064	0	0	0	3	0	8	1	10	0	0
Lot 29211001	0	0	0	0	0	0	0	0	0	0
Lot 29210011	0	0	0	0	0	0	0	0	0	0
Lot 4017064+hydrocortisone										
Sodium hemisuccinate (10^{-7})	41	>100	>100	>100	41	>100	>100	>100	>100	31
+Methylprednisolone sodium hemisuccinate (10^{-7})	50	>100	>100	>100	61	>100	>100	>100	>100	41
+Cortisone acetate (10^{-7})	51	>100	>100	>100	13	>100	>100	>100	>100	50
+Dexamethasone phosphate (10^{-7})	10	>100	>100	>100	61	>100	>100	>100	>100	40
+Prednisolone sodium phosphate (10^{-7})	8	>100	41	38	22	17	51	>100	>100	30
+Testosterone enanthate (10^{-7})	0	0	0	0	0	6	0	0	0	0
+Oestradiol valerate (10^{-7})	0	0	0	0	0	8	0	0	0	0
+d-Aldosterone (10^{-7})	10	30	43	>100	30	6	>10	>10	>10	40
+Testosterone (10^{-7})	0	0	0	0	0	10	0	0	0	0
+Androstendione (10^{-7})	0	0	0	0	0	5	0	0	0	0
+Corticosterone (10^{-7})	0	20	30	>100	20	8	40	>100	>100	30
+Dehydroepiandrosterone sulphate (10^{-7})	0	0	0	0	0	10	0	0	0	0
+β-Oestradiol (10^{-7})	0	0	0	0	0	6	0	0	0	0
+Oestrinol (10^{-7})	0	0	0	0	0	5	0	0	0	0
+Oestrone (10^{-7})	0	0	0	0	0	8	0	0	0	0
+Progesterone (10^{-7})	0	0	0	0	0	10	0	0	0	0
+Pregnenolone (10^{-7})	0	0	0	0	0	9	0	0	0	0

Each week drugs (Sigma) were dissolved in ethanol or acetone according to published procedures in 1,000-fold concentrations and added in 0.01-ml volumes to each 10-ml culture flask. Results are the mean for at least three cultures in each set of conditions. At least 10 microscopic fields were scored for each culture. The cortisol concentration measured by radioimmunoassay in Manchester horse serum ($10.0 \mu\text{g ml}^{-1}$) did not differ significantly from that found in six lots of Rockville horse, fetal calf or goat serum (range 2.5 – $25 \mu\text{g ml}^{-1}$). No difference was detected in corticosterone or *d*-aldosterone concentrations. These levels reflect total protein-bound and free corticosteroid. The biologically active unbound fraction was increased variably by addition of free steroid⁽¹⁵⁾. Results are for NIH/Swiss mouse marrow.

block of insulin receptors³. Furthermore, adrenal corticosteroids directly or indirectly (through catecholamines) accelerate lipolysis of triglyceride to free fatty acid in non-marrow adipocytes, in contrast to the effect on marrow reported here^{7,8}. Thus, my data showing no insulin preincubation block of corticosteroid induced-lipogenesis in marrow preadipocytes provide strong evidence for the uniqueness of the marrow regulatory pathway. I have also observed corticosteroid-inducible lipogenesis in rat and human marrow. It will be interesting to determine whether marrow lipogenesis, once stimulated, requires the same metabolic pathways as the insulin-dependent epididymal adipocyte and the 3T3-L1 cell line^{3,4,9}. The increased sensitivity of a distinct corticosteroid-dependent population of marrow preadipocytes in mutant obese-diabetic mice indicates that the gene for obesity¹⁰⁻¹⁴ affects lipid metabolism in multiple cell phenotypes.

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Enzyme replacement using liposome carriers in feline G_{M1} gangliosidosis fibroblasts

CURRENT emphasis in research on lysosomal storage diseases is directed towards enzyme replacement therapy (ERT) for these inherited enzyme deficiencies¹⁻³. The rationale for ERT is based on the assumption that endocytosed enzyme would enter lysosomes and function in the degradation of accumulated substrates. However, inactivation of enzyme, immunological reactions and low cellular uptake have prevented successful application of this promising approach to therapy. Circumvention of these limitations requires the use of carriers which protect and stabilise replacement enzyme while enhancing uptake and subcellular distribution²⁻⁵. We report here the use of lipid vesicles (liposomes)⁶⁻⁹ as carriers for replacement enzyme in feline G_{M1} gangliosidosis fibroblasts which have deficient β -galactosidase (β -gal) activity and lysosomal storage of glycopeptides⁹⁻¹¹. Our results demonstrate increased β -gal activity and corresponding catabolism of stored glycopeptides in feline G_{M1} gangliosidosis fibroblasts treated with liposomes containing β -gal.

Fibroblast cultures derived from skin of normal cats and cats with G_{M1} gangliosidosis were maintained in McCoy's 5a medium with 20% fetal calf serum¹². β -Galactosidase activity was determined with the fluorogenic substrate, 4-methyl-umbelliferyl- β -D-galactoside (4-MU- β -galactoside) (1 unit = 1 nmol substrate cleaved h⁻¹)^{10,13}, and protein was estimated by the

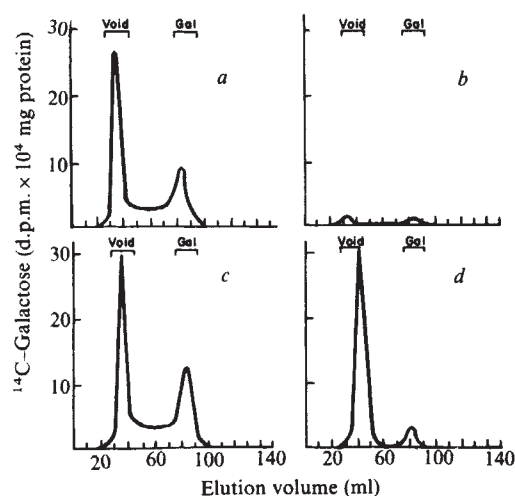


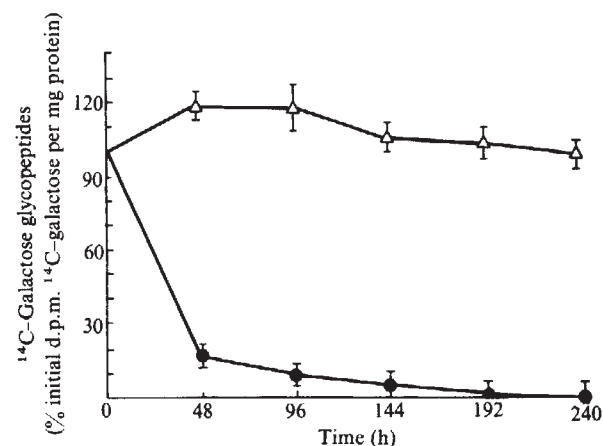
Fig. 1 Elution patterns of ¹⁴C-GP from a 0.9×100 cm BioGel P10 column. Homozygous dominant fibroblasts at: a, end of pulse and b, 240 h after pulse, and homozygous recessive fibroblasts at: c, end of pulse and d, 240 h after pulse. (4 ml fractions.)

method of Lowry¹⁴. Partially purified β -gal was obtained by concanavalin A chromatography of acetone-precipitated normal feline liver homogenate¹⁰. Large multilamellar liposomes (1,000-6,000 Å) containing β -gal [L(β -gal)] or thorium dioxide [L(thor)] were prepared from phosphatidylcholine, dicetyl phosphate and cholesterol in a molar ratio of 7:2:1 (refs 5-8). Emulsification of the lipids in 6 ml of aqueous β -gal or thorium dioxide in a bath-type sonicator produced liposomes containing 11-13% of the total aqueous solute.

Entrapment of solutes within aqueous interstices of liposomes was confirmed by chromatographic separation of liposomes from untrapped solute and by demonstration of latency of entrapped solutes⁸. The integrity of the liposomes during incubation with fibroblasts was demonstrated by similar analysis of spent culture fluid⁸. In all cases, there was retention of entrapped solutes within chromatographic fractions containing liposomes. Entrapped β -gal was shown to be latent until the liposomes were lysed with Triton X-100.

Normal and G_{M1} gangliosidosis fibroblasts were incubated with 2 μ Ci ¹⁴C-galactose to label the endogenous glycopeptide pool^{11,15}. The degree of labelling and metabolic fate of ¹⁴C-galactose-labelled glycopeptides (¹⁴C-GP) were evaluated at 48-h intervals by chromatographic separation on BioGel P10. The elution patterns of ¹⁴C-GP from normal and G_{M1}

Fig. 2 Dynamics of ¹⁴C-GP metabolism in (●) homozygous dominant and (Δ) homozygous recessive fibroblasts following a 48-h pulse with 2 μ Ci ¹⁴C-galactose per mg protein.



gangliosidosis fibroblasts are shown in Fig. 1. Total ^{14}C -GP was expressed for each interval as d.p.m. ^{14}C per mg protein in fractions 5–11. In normal fibroblasts there was initial labelling of ^{14}C -GP followed by rapid catabolism and clearance of ^{14}C , reflecting normal β -gal activity. However, in G_{M1} gangliosidosis cells β -gal deficiency was expressed by retention of ^{14}C -GP (Fig. 2).

Uptake and intracellular fate of liposomes were evaluated by electron microscopy of fibroblasts which had been incubated with L[thor]. Monolayers were incubated with 2,000 μg liposomal lipid for 6 h, washed three times, detached with 0.25% trypsin, centrifuged and prepared for electron microscopy¹⁶. The intracellular accumulation of thorium dioxide within heterophagosomes and membranous residual bodies is shown in Fig. 3.

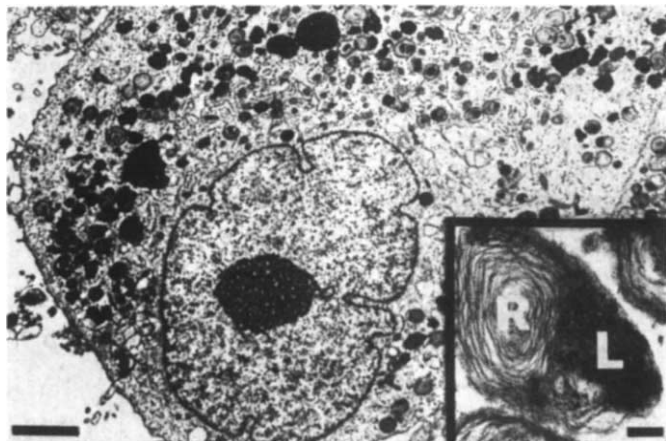


Fig. 3 Electron micrograph of a G_{M1} gangliosidosis fibroblast following incubation with L[thor] demonstrating the presence of thorium dioxide within the lysosomal apparatus of the cell (bar = 1.0 μm). Insert illustrates fusion between a secondary lysosome (L) containing Thorotrast and a membranous residual body (R) characteristic of G_{M1} gangliosidosis fibroblasts (bar = 0.1 μm).

The efficacy of ERT was evaluated by labelling G_{M1} gangliosidosis fibroblasts with ^{14}C -galactose as described above. After 48 h, each monolayer was treated with either L[β -gal] containing 3,000 units of β -gal activity and 2,000 μg liposomal lipid or 3,000 units of untrapped β -gal. Control samples consisted of untreated fibroblasts, fibroblasts treated with liposomes containing buffer, or fibroblasts treated with liposomes containing buffer plus untrapped β -gal (3,000 units). Treatments were repeated and samples collected in 48-h intervals. Changes in ^{14}C -GP were monitored as described above, and changes in enzyme activity were monitored by assay of cell lysates. Treatment with either unencapsulated β -gal or any of the control treatments had no effect on ^{14}C -GP storage. However, treatment with L[β -gal] resulted in clearance of >80% of ^{14}C -GP and increased β -gal activity to 70% of normal, reflecting uptake of approximately 10% of the total enzyme activity presented in liposomes (Fig. 4).

Characterisation of this *in vitro* system, in which the dynamics of naturally occurring lysosomal storage can be quantitated, provides a unique model for precise assessment of ERT. Endogenous storage material in feline G_{M1} gangliosidosis fibroblasts seems to be similar to that found in human G_{M1} gangliosidosis fibroblasts¹¹. This glycopeptide is structurally similar to storage material found in hepatocytes of human and feline G_{M1} gangliosidosis^{17,18}, suggesting a potentially meaningful analogy between *in vitro* and *in vivo* therapeutic trials.

The ultrastructure of cells treated with L[thor] confirmed the lysosomotropic effect of liposomes. Although fusion of liposomes with primary lysosomes has been documented previously⁸, this study clearly demonstrates fusion of liposomes with membranous residual bodies characteristic of many

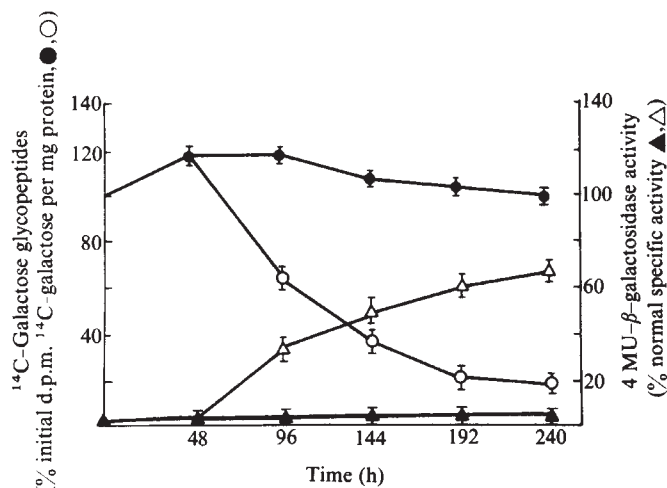


Fig. 4 Effect of enzyme replacement therapy on ^{14}C -GP storage and β -gal activity of G_{M1} gangliosidosis fibroblasts treated with: ●, ▲, unencapsulated β -gal, or, ○, △, L(β -gal). Differences in ^{14}C -GP storage and β -gal activity of all control samples and samples treated with unencapsulated β -gal were not statistically significant.

lysosomal storage diseases. This observation is important evidence supporting the potential value of liposome carriers in the prevention and reversal of lysosomal storage.

Failure of fibroblasts treated with unencapsulated enzyme to increase enzyme activity or degrade accumulated substrate supports the contention that a carrier is needed to introduce therapeutic quantities of hydrolytically active enzymes. Furthermore, increased levels of appropriate enzyme activity and enhanced degradation of endogenously accumulated substrate clearly demonstrate the value of liposomes as carriers of replacement enzyme in the correction of a metabolic defect in fibroblasts.

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Calcium and magnesium ions and the regulation of multiplication in normal and transformed cells

THE relative importance of Ca^{2+} and Mg^{2+} in the regulation of cellular multiplication and alteration of that regulation by viral transformation has recently become a subject of considerable interest¹⁻⁵. Extracellular Ca^{2+} and Mg^{2+} both have effects on metabolic parameters and macromolecular synthesis in cultured cells. Mg^{2+} , because of its substrate level intracellular concentration and its role in macromolecular synthesis and intracellular transphosphorylation reactions, has been suggested as a possible coordinate regulator of the positive pleiotropic response, a coordinated array of reactions associated with initiation of DNA synthesis and multiplication in cultured cells⁶⁻⁹. Ca^{2+} , on the other hand, has been suggested as a short term regulator of cell growth and function, largely on the basis of its tightly regulated low cytoplasmic concentration^{10,11}. In this report, we describe a kinetic analysis of the effects of extracellular concentrations of Ca^{2+} and Mg^{2+} on survival and multiplication of normal and transformed human cells in culture. We have found that cellular

multiplication rate (number of population doublings per unit time) and also cellular 'survival rate' (percentage survival divided by time) can be substituted in place of reaction rate in classical enzyme kinetic formulae (Fig. 1). Both responses exhibit saturation kinetics, with a transition from first order response to zero order, as the concentration of calcium, magnesium, or any other essential component of the culture medium is increased. Rate plotted against concentration yields the expected right rectangular hyperbola described by the Henri-Michaelis-Menten formulation, and the expected linear relation when plotted by the Lineweaver-Burke or van Hofstee methods. Linear regression of the Lineweaver-Burke double reciprocal plot yields from its intercepts a maximal rate for multiplication or survival (equivalent to $V_{\max}$ in classical enzyme kinetics) and a Michaelis-Menten constant (K_m), equal to the substrate (or ligand) concentration yielding a half maximal rate of multiplication or survival.

In order to apply Michaelis-Menten kinetic analyses to cellular multiplication and survival, we have made the following assumptions: (1) each substrate or ligand (S) interacts with sites on or in each cell (C) to form complexes (CS), operationally equivalent to the ES complex of first order enzymatic reactions; (2) although each cell may have many sites that form complexes with S, the interactions between these sites and S are such that

Fig. 1 Multiplication response of normal and transformed human lung fibroblasts to Mg^{2+} . *a*, Colony size: previously described procedures for clonal growth and measurement of colony size¹²⁻¹⁴ were used, with the modifications described below. The basal medium was MCDB 105 (ref. 14) with pantothenate supplied as a sodium salt (Sigma), and with calcium chloride and magnesium sulphate omitted. For these experiments the basal medium was supplemented with 1.0×10^{-3} M ultrapure grade $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$ (Alfa) and $250 \mu\text{g ml}^{-1}$ dialysed fetal bovine serum protein (FBSP)¹⁵. Ultrapure grade $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Alfa) was added as indicated. Polylysine-coated tissue culture Petri dishes were used for N-HLF¹⁶. Uncoated dishes were used for SV-HLF, whose multiplication is either unaffected or slightly inhibited by the basic polymer. Each 60-mm dish was inoculated with 100 cells and incubated for 14 d. ●, Normal cell data for MRC-5 (ATCC CCL 171) at passage 20. Similar results have also been obtained with WI-38 and FLOW-2000 cells. ○, Transformed cell data for SV40 virus transformed WI-38 (Subline 2RA, ATCC CCL 75.1). The size of the five largest colonies per plate was measured photometrically with an aperture of 7 mm for N-HLF and 4 mm for SV-HLF as previously described¹². We have shown experimentally by trypsinisation and direct cellular counting with a Coulter counter that the photometric measurement averaged over the five largest colonies per dish is linearly related to the total number of cells per Petri dish (the sum of all colonies) and to the number of cells per colony in the colonies that are measured. Thus, the photometric measure can be used as an accurate indicator of total multiplication during the incubation period. *b*, multiplication rate: During the observation periods used here (7–14 d), the increase in colony size is essentially exponential. The increase in $\log C$ against t , where C is colony size and t incubation period, measured either by photometric or direct counting methods has a linear correlation coefficient $> +0.93$ for any given set of experimental conditions during that time period. Experimental data were normally collected after 14 d of incubation to minimise error in measurement of very small colonies. Colony size in photocell units was converted to multiplication rate by the formula $r = 3.32(\log_{10} C_t - \log_{10} C_0)/t$ where r = cell generations (population doublings) per d, t = incubation period in d, 3.32 = reciprocal $\log_{10} 2$, C_t = colony size in photocell units at the end of t d, and C_0 = colony size in photocell units at time zero. C_0 is a general background constant determined by linear regression to $t = 0$ of a plot of $\log_{10} C$ against t . For N-HLF, $C_0 = 0.080$ photocell units, and for SV-HLF, $C_0 = 0.11$ photocell units. The experimentally determined multiplication rates (r) for the various concentrations of Mg^{2+} have been fitted to a right rectangular hyperbola according to the Henri-Michaelis-Menten formulation. *c*, Lineweaver-Burke double reciprocal plot of the data from *b*. Values for the Michaelis-Menten constant (K_m) corresponding to the concentration of Mg^{2+} yielding a half maximal multiplication rate, and for $r_{\max}$ are obtained from the intercepts as shown. The best linear fit to the data was determined by 'least squares' estimate. Experiments resulting in regression lines with linear correlation coefficient $< +0.80$ were rejected for use in these analyses. Errors in K_m and $r_{\max}$ were estimated by Gaussian analysis of variance based on single data points which were means of triplicate determinations. Standard errors between triplicate individual determinations are indicated in *a* and *b* for N-HLF. For N-HLF, $K_m = 4.8(\pm 1.6) \times 10^{-5}$ M, $r_{\max} = 0.492 \pm 0.055$, and for SV-HLF, $K_m = 4.2(\pm 1.0) \times 10^{-6}$ M, $r_{\max} = 0.425 \pm 0.010$ at $P = 0.05$. The transformation of data on colony size and incubation time to a rate function distinguishes this analysis from previous studies seeking to apply enzyme reaction rate kinetics to cellular growth response, in which the fraction of cells synthesising DNA was measured rather than actual cellular multiplication rates¹⁷.

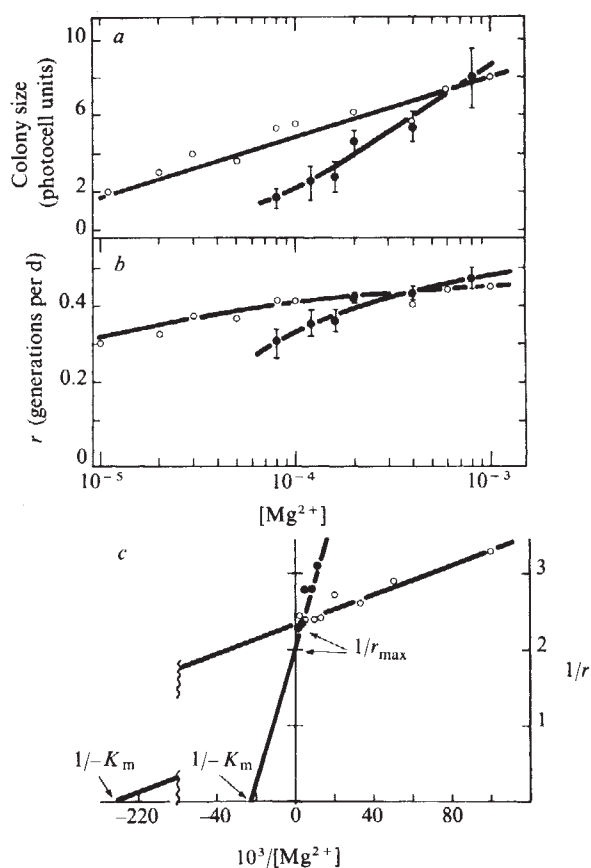


Table 1 Interaction of Mg^{2+} and Ca^{2+} in multiplication of normal and transformed human lung fibroblasts

Mg^{2+} (M)	N-HLF		SV-HLF	
	$K_m^{Ca^{2+}}$ ($mol\ l^{-1}$)	r_{max} (cell generations per d)	$K_m^{Ca^{2+}}$ ($mol\ l^{-1}$)	r_{max} (cell generations per d)
10^{-3}	$1.2(\pm 0.70) \times 10^{-5}$ NS	0.433 ± 0.025 ($P < 0.001$)	$1.1(\pm 0.64) \times 10^{-5}$ ($0.05 < P < 0.10$)	0.412 ± 0.025 ($0.02 < P < 0.05$)
10^{-4}	$1.5(\pm 1.25) \times 10^{-5}$	0.314 ± 0.039	$1.7(\pm 1.4) \times 10^{-5}$	0.398 ± 0.022
Ca^{2+} (M)	$K_m^{Mg^{2+}}$	r_{max}	$K_m^{Mg^{2+}}$	r_{max}
	($mol\ l^{-1}$)	(cell generations per d)	($mol\ l^{-1}$)	(cell generations per d)
10^{-3}	$3.9(\pm 0.32) \times 10^{-5}$ ($P < 0.001$)	0.463 ± 0.010 NS	$7.8(\pm 0.98) \times 10^{-7}$ ($P < 0.001$)	0.404 ± 0.010 ($P < 0.001$)
10^{-4}	$1.1(\pm 0.80) \times 10^{-4}$	0.481 ± 0.08	$3.3(\pm 0.83) \times 10^{-6}$	0.351 ± 0.022

Kinetic parameters for the stimulation of multiplication of N-HLF (WI-38, passage 20) and SV-HLF by Ca^{2+} and Mg^{2+} were determined for each ion as a function of the concentration of the other. The procedures were the same as described in Fig. 1. The concentrations of both ions were varied within a single series of assays using the same stock cultures, basal media and FBSP. Values $\pm 95\%$ confidence limits are indicated. The probability (P) that the difference between values is due to chance was determined by Student's t test and is indicated between rows. NS, not significant or $P > 0.10$.

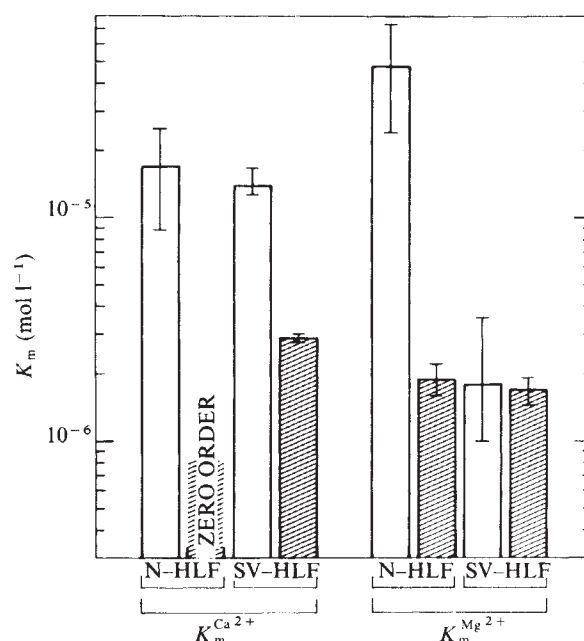
formation of CS can be treated as a first order function of S; (3) for each substrate or ligand (S), the rate of cellular multiplication (or survival) when all other substrates or ligands are constant is a first order function of the concentration of the CS complex; (4) the concentration of S is large relative to the concentration of sites in or on the cells, such that formation of CS does not significantly alter S; (5) consumption of S is sufficiently small that its concentration is not altered significantly and rates can be considered uniform throughout the assay period; (6) the rate of equilibration among cells, S, and CS complex is rapid relative to the rate of cellular multiplication or other processes that affect the concentration of CS; and (7) in the case of cellular multiplication, it is also necessary to assume that all daughter cells are identical to the parental cells, and cells remain in an exponential pattern of multiplication with no significant differentiation or other forms of withdrawal from the cell cycle.

The experimental system used in these studies is clonal growth of human diploid fibroblast-like cells derived from fetal lung (WI-38, MRC-5 and FLOW-2000 are interchangeable and are referred to as N-HLF) and a comparable transformed line (SV40 transformed WI-38, referred to as SV-HLF). An optimised medium (MCDB 105 (ref. 14)) was used which has each of its components adjusted to the centre of the concentration range yielding a zero order response¹²⁻¹⁴. The volume of medium per cell is large, and direct analysis of used media has verified that the concentrations of essential components do not change significantly during the assay period. Thus, the system seems to satisfy the assumptions made, as will be described elsewhere.

For N-HLF, both Ca^{2+} and Mg^{2+} are absolute requirements for multiplication, even when the other is present in saturating amounts. The requirements for multiplication are much greater than those for survival (Fig. 2). In these experimental conditions, the $K_m^{Mg^{2+}}$ for survival is only 5–6% of that for multiplication, and no effect of Ca^{2+} on survival rate can be demonstrated (zero order response). However, we cannot conclude that no Ca^{2+} is needed for survival, as atomic absorption spectrometry shows that the 'calcium-free' test medium contains $1.5 (\pm 0.12) \times 10^{-5}$ M calcium as a contaminant. The comparable 'magnesium-free' medium contains $8.6 (\pm 0.46) \times 10^{-6}$ M magnesium. These amounts are not due to contaminants in the high purity calcium and magnesium salts (each of which contains less than 2 p.p.m. of the other) or in the dialysed serum protein ($250\ \mu g\ ml^{-1}$) used in the assay.

Transformation of N-HLF with SV40 reduces the $K_m^{Mg^{2+}}$ for multiplication to that required for maintenance of either the normal or transformed cell (Fig. 2). In contrast, the amount of extracellular Ca^{2+} required for a half maximal rate of multiplication ($K_m^{Ca^{2+}}$) is changed little by transformation. However, some multiplication of SV-HLF occurs in the absence of added calcium, which could reflect either the background Ca^{2+} contamination or a 'basal' growth rate that does not require

Fig. 2 K_m values for multiplication and survival responses of N-HLF and SV-HLF to Ca^{2+} and Mg^{2+} . K_m values were determined as described for Fig. 1. Responses to Ca^{2+} and Mg^{2+} were measured with $250\ \mu g\ ml^{-1}$ FBSP and 1.0×10^{-3} M of the other ion. Survival rate was determined by incubating 100 cells per 60-mm Petri dish in 5.0 ml of a medium that is not permissive for multiplication. After 4 d, the medium was replaced with a complete growth medium and the dishes were incubated for a further 10 d after which the colonies were fixed, stained and counted. The survival media were similar to the growth media described for Fig. 1, except that in place of FBSP they contained $80\ \mu g\ ml^{-1}$ delipidated FBSP¹⁸. Delipidation of FBSP removes or inactivates one or more factors that are essential for multiplication of both types of cells¹⁹. N-HLF will survive in MCDB 105 without proliferation in the total absence of serum-derived factors^{13,14}. However, survival of SV-HLF in the same medium requires serum-derived factors that are not inactivated by delipidation. Delipidated serum was used with both types of cells for uniformity. Survival rate (number of survivors divided by days in survival medium) was measured as a function of concentration of Ca^{2+} or Mg^{2+} (with the other at 1.0×10^{-3} M) and K_m values for survival were determined from Lineweaver-Burke double reciprocal plots. White bars, multiplication; shaded bars, survival. Each value represents mean $\pm$ s.e.m. of three independent determinations using different stock cultures, media and FBSP preparations. Under the experimental conditions, Ca^{2+} has no effect on survival rate of N-HLF, possibly because of a low background level of calcium in the basal medium (see text).



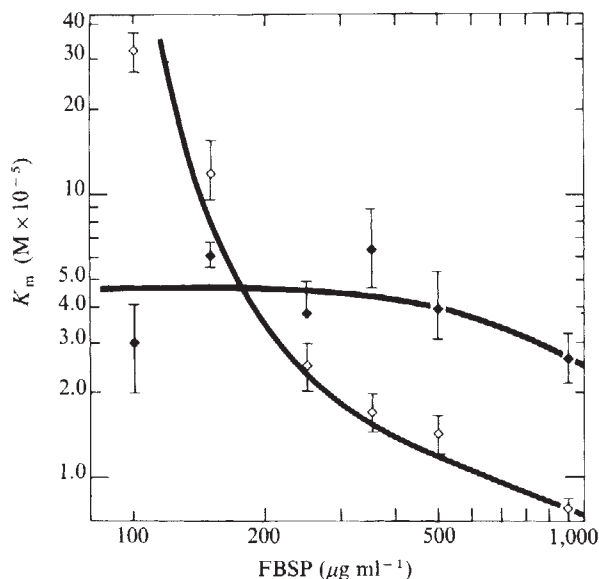
Ca^{2+} . Additional experiments in a cleaner background medium are needed to distinguish between these possibilities. In contrast to N-HLF, SV-HLF cells exhibit an easily measured survival response to Ca^{2+} which can be extrapolated to give a $K_m^{\text{Ca}^{2+}}$ of about 2.9×10^{-6} M. The $K_m^{\text{Mg}^{2+}}$ for survival is essentially unchanged by transformation.

In an attempt to determine whether Ca^{2+} or Mg^{2+} exerts the most proximal effect on cellular multiplication, we determined both K_m and $r_{\max}$ for each ion as a function of the other (Table 1). For N-HLF, the amount of Mg^{2+} in the medium has little effect on the amount of Ca^{2+} needed for a half maximal response ($K_m^{\text{Ca}^{2+}}$), but has a direct effect on the maximal growth response to calcium that can be obtained ($r_{\max}^{\text{Ca}^{2+}}$). Ca^{2+} , however, has no effect on the maximal growth that can be obtained ($r_{\max}^{\text{Mg}^{2+}}$), but low levels of calcium significantly increase the amount of magnesium needed for the response ($K_m^{\text{Mg}^{2+}}$). We interpret these results, in agreement with Rubin⁵, as indicating that for normal cells the role of Mg^{2+} is more proximal to the intracellular events that determine $r_{\max}$ for cellular multiplication than are those processes directly affected by Ca^{2+} .

In transformed cells, $r_{\max}$ values that can be achieved are affected less by Mg^{2+} , but seem to be dependent on Ca^{2+} . This observation is consistent with the interpretation that Mg^{2+} does not have a specific regulatory role in the multiplication of transformed cells and suggests that Ca^{2+} has the most proximal role in the multiplication of such cells. These striking and complex differences between normal and transformed HLF show the need for further investigation of the relationship.

In the presence of saturating amounts of Ca^{2+} and Mg^{2+} (10^{-3} M each), multiplication, but not survival, of N-HLF is dependent on serum macromolecules whose role is presumed to be regulatory¹⁴. To investigate the interactions among Ca^{2+} , Mg^{2+} , and serum-derived regulatory macromolecules, we determined K_m for both ions as a function of serum protein (FBSP) concentration in the medium (Fig. 3). K_m values for both ions tend to rise as FBSP concentration is reduced. However, at very low levels of FBSP, $K_m^{\text{Ca}^{2+}}$ rises very steeply, whereas $K_m^{\text{Mg}^{2+}}$ levels off, suggesting that Ca^{2+} may be the ion that interacts the more directly with serum macromolecules that exert multiplication-stimulating effects on normal cells.

Fig. 3 Effect of FBSP on apparent K_m for Ca^{2+} and Mg^{2+} for multiplication of N-HLF. Multiplication rate responses to Ca^{2+} and Mg^{2+} with the other at 1.0×10^{-3} M were measured at each of the indicated levels of FBSP and K_m values were determined in a single experiment as described in Fig. 1. The N-HLF stock culture used here was MRC-5 (passage 20). Vertical bars, s.e. due to the regression analysis. $\diamond$, $K_m^{\text{Ca}^{2+}}$; $\blacklozenge$, $K_m^{\text{Mg}^{2+}}$.



From these data we propose that (1) Ca^{2+} and Mg^{2+} have equally important roles in regulation of cellular multiplication that go beyond support of attachment and survival of non-proliferating cells; (2) transformation causes a selective loss of the regulatory role of Mg^{2+} , but not Ca^{2+} , in cellular multiplication; (3) in normal cells, Mg^{2+} has its regulatory effect on a process more proximal to the intracellular events of cellular replication than those processes affected directly by Ca^{2+} ; (4) in normal, but not transformed cells, the regulatory effect of Ca^{2+} is primarily mediated through Mg^{2+} -dependent processes; (5) the role of Ca^{2+} is more proximal than that of Mg^{2+} to the action of regulatory macromolecules from serum in the chain of events which ultimately determine multiplication rate of normal cells.

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Does Ca^{2+} cause fusion or lysis of unilamellar lipid vesicles?

MEMBRANE fusion is the central event in biological processes as diverse as secretion, fertilisation, endocytosis and pathological cell fusion, but as yet its mechanism is poorly understood¹. To elucidate this mechanism, bilayer phospholipid vesicles have been used extensively as models for interacting biomembranes^{2–5}. As a result of studies involving differential scanning calorimetry and freeze-fracture electron microscopy, it has been suggested that vesicles made from acidic phospholipids can fuse when exposed to divalent cations^{3,6}. For phosphatidylserine vesicles, the 'critical' Ca^{2+} concentration for membrane fusion seems to be ~ 1 mM, at which concentration a drastic increase in the permeability of the vesicle membranes has also been reported³. The techniques used to demonstrate 'fusion' in this system do not exclude molecular mixing of interacting membranes by mechanisms other than genuine fusion. In the latter case, the membrane-bounded internal compartments of two separate vesicles must become confluent in a process which transfers the contents of the initial vesicles to the resulting vesicle. It is possible, however, that Ca^{2+} may cause rupture of the lipid vesicles, with subsequent reassembly of the membrane fragments to form larger non-vesicular structures. If this is occurring, bilayer vesicles are poor paradigms for biological

Table 1 Leakage of ^3H -sucrose from liposomes

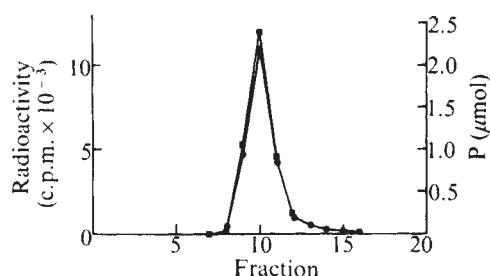
	Ca^{2+}	Mg^{2+}
Control	—	94.6
0.1	88.6	94.1
1	37.9	88.5
10	1.3	13.7

% of total radioactivity remaining associated with liposomes after incubation with divalent cations (mM) for 1 h. The higher divalent cation concentrations caused an increase in the size of lipid particles in the suspensions, preventing their passage through a Sephadex column. A Millipore filter (0.8 μm pore diameter) was mounted above the column and the sample to be eluted was pipetted on to it. The column eluant was treated similarly. Consequently, any lipid particles below the size limit of the filter appeared in the void volume and the eluant effectively washed any free sucrose from the larger particles trapped on the Millipore. Radioactivity remaining associated with the Millipore was counted after dissolving the filter in a Triton X-100-based scintillation mixture. A quenching correction was applied.

membrane fusion. One may predict the likelihood of vesicle fusion by calculating their energy of interaction due to electrostatic repulsion and electrodynamic attraction⁷. This approach shows that the addition of 1 mM Ca^{2+} to a suspension of phosphatidylserine vesicles is unlikely to reduce electrostatic repulsion between liposomes sufficiently for any but a fraction of the smallest ones to approach to 0.8 nm separation. This finding suggests that vesicles cannot approach closely enough to fuse and supports the notion that Ca^{2+} may initiate vesicle lysis and reassembly. We report here experimental support for vesicle lysis, based on the redistribution of a marker for the aqueous phase between vesicles and the external compartment on exposure to divalent cations.

Sonicated unilamellar vesicles were prepared from phosphatidylserine (Lipid Products, grade 1, >99% pure; stored before use in chloroform under nitrogen at -20°C) according to the methods of Papahadjopoulos *et al.*^{3,6,8-11}. The vesicles (10 μmol of lipid per experiment) were suspended in 2 ml of 100 mM NaCl containing 20 μCi of ^3H -sucrose (Amersham). One hour after sonication they were separated from untrapped sucrose by elution of the suspension through a Sephadex G-50 column. One 10-drop fraction (0.92 ml) representing the centre of the lipid peak was collected. This fraction was then exposed to a known final concentration of Ca^{2+} or Mg^{2+} for a further hour, after which the proportion of the total sucrose remaining associated with the lipid was estimated by eluting the sample from a second Sephadex G-50 column (the eluting medium contained divalent cations at the same concentration as in the sample) and determining the amount of radioactivity in each fraction using a liquid-scintillation counter. These amounts were then compared with the location of the lipid peak in the total elution profile, as determined by phosphate analysis (modification of the method described by Allen¹²).

Fig. 1 Elution of phosphatidylserine vesicles containing ^3H -sucrose from a Sephadex G-50 column (300 $\times$ 10 mm). The location of the lipid peak (■) was determined by phosphate analysis. Radioactivity (●) is expressed as c.p.m. $\times 10^{-3}$.



In the absence of divalent cations, most of the marker remained associated with the vesicles 1 h after the first gel filtration (Fig. 1). Exposure to increasing concentrations of Ca^{2+} or Mg^{2+} , however, brought about a progressive release of ^3H -sucrose from the liposomes (Table 1). Ca^{2+} was more effective than Mg^{2+} , a finding in agreement with the work of others¹³.

If this release was the result of a divalent cation-induced increase in vesicle membrane permeability, one would expect to be able to incorporate radioactive sucrose into previously unlabelled vesicles by exposing them to divalent cations. To test this, sonicated vesicles were prepared in the absence of ^3H -sucrose. 20 μCi of this radiochemical were then added to the samples immediately after sonication and the uptake of radioactivity into vesicles was assessed following exposure of the samples to Ca^{2+} or Mg^{2+} .

Without divalent cations, only 0.007% of the total marker became associated with the vesicles during the incubation (Fig. 2). Thus, very little sucrose had leaked into vesicles or been adsorbed on to their membranes. Furthermore, the presence of Ca^{2+} or Mg^{2+} hardly affected this uptake (Table 2). It might be argued that the results in Table 2 are artificially low due to the loss of vesicle-incorporated sucrose during the separation of the lipid from the external compartment by the filtration procedure (see legend to Table 1). To exclude this possibility, vesicles were prepared without ^3H -sucrose. This tracer was then added to the external compartment, followed by Ca^{2+} to a final concentration of 10 mM. After 1 h, non-incorporated sucrose was removed by gel filtration, eluting with divalent cation-free 25 mM EGTA.

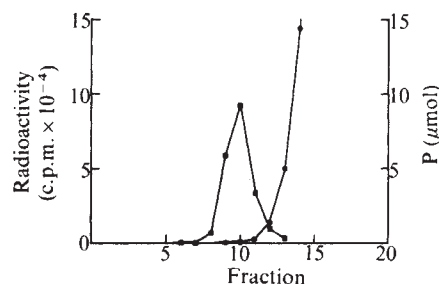


Fig. 2 Elution of a suspension of phosphatidylserine vesicles with ^3H -sucrose in the external aqueous compartment from a Sephadex G-50 column. Symbols as in Fig. 1.

Chelating agents can halt the Ca^{2+} -induced leakage of $^{22}\text{Na}^+$ and $^{42}\text{K}^+$ from phosphatidylserine liposomes¹³; thus, any ^3H -sucrose which had diffused into vesicles during incubation with 10 mM Ca^{2+} would not be washed out during elution. Only 0.002% of the total sucrose became associated with the lipid, implying that the values in Table 2 are not erroneously low.

Permeability coefficients for phosphatidylserine membranes may be calculated from Tables 1 and 2. In the 10 mM Ca^{2+} case, the ratio of these coefficients for release/uptake is 144:1 (1,170:1 in the EGTA version of the experiment). Divalent cations therefore seem to be able to bring about leakage of ^3H -sucrose from phosphatidylserine vesicles but not into them. It is unlikely that Ca^{2+} or Mg^{2+} is able to open a unidirectional pore in membranes as relatively simple as those under consideration. The most probable explanation for these findings is

Table 2 Leakage of ^3H -sucrose into liposomes

	Ca^{2+}	Mg^{2+}
Control	—	0.007
0.1	0.019	0.038
1	0.004	0.032
10	0.016	0.013

% of total radioactivity becoming associated with liposomes after incubation with divalent cations for 1 h. The % of total radioactivity that might be expected to be captured if the liposomal internal space equilibrated with the external space = 0.486. Divalent cation concentrations are in mM.

therefore that divalent cations bring about the complete rupture of phosphatidylserine vesicles, with production of structures which have lost the form of closed vesicles. This conclusion suggests the need for caution in interpreting vesicle 'permeability increases' under the influence of agents other than Ca^{2+} and Mg^{2+} . Regarding membrane fusion, it seems that the Ca^{2+} -phosphatidylserine system is an inappropriate model for biological membrane interaction, as the final product of the reaction between these components is not vesicular, although some initial fusion may occur on addition of Ca^{2+} to the suspension. Identification of the factors essential to fusion must await the development of a reliable assay for the occurrence of this process in artificial membrane systems.

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Uptake of charged lipid vesicles by isolated tomato protoplasts

LIPID vesicles act as carriers of biologically active materials into animal cells¹⁻³. A similar role might be envisaged for vesicles, for example, in the introduction of foreign DNA into plant protoplasts in genetic modification experiments⁴, or in the initiation of virus infection in plant protoplasts⁵. The amount of DNA which enters fed protoplasts is difficult to determine as is the multiplicity of virus which initiates infection in the poly-L-ornithine (PLO) (ref. 5) and polyethylene glycol (PEG) (ref. 6) procedures. To overcome these limitations of existing methods for the introduction of materials into protoplasts, I report here a vesicle system with the potential to fuse with the negatively charged plasmalemma⁷. Single membraned ('hollow') vesicles were considered more suitable to combine membrane instability with large 'load' volume, required to facilitate carriage of large molecules/virus particles into the protoplast and to release their content synchronously within.

Tomato protoplasts were isolated as previously described⁸. Positively charged vesicles were prepared as follows: equal volumes of 10 μM lecithin and 1 μM stearyl amine were dissolved in chloroform-methanol (4:1, v/v) and evaporated to dryness⁹. The residue was resuspended at 2.5 mg ml^{-1} in phosphate buffered mannitol (PBM, 12% w/v mannitol in 50 mM sodium phosphate buffer, pH 7.0) containing 0.01% w/v fluorescein diacetate and shaken vigorously by hand to give vesicles of about 0.002-0.006 μm diameter. These were loaded with fluorescein diacetate to facilitate study. The vesicles were collected by centrifugation at 50 g for 10 min. Vesicles were washed by resuspension, recentrifugation in PBM. Small vesicles remained in the supernatant and were discarded.

Protoplast and vesicle counts were made and the populations mixed in the ratio of 1:1, protoplasts:vesicles, in a volume of 1 ml (1×10^6 protoplasts) of PBM. Samples were examined

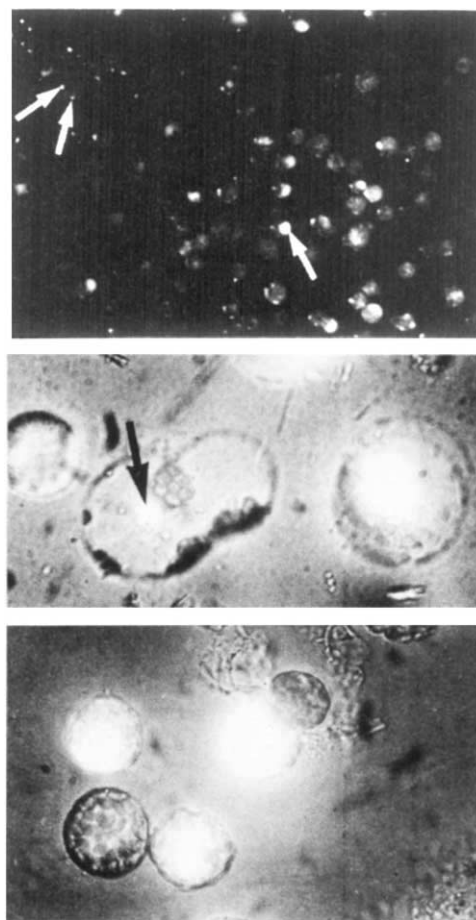


Fig. 1 Uptake of charged vesicles by isolated tomato protoplasts. Top, binding of vesicles to the plasmalemma, arrows indicate binding of one or more vesicles per protoplast. Middle, uptake of vesicles into the central vacuole, indicated by arrow. In the adjacent protoplast, the vacuole is releasing its contents. Bottom, some protoplasts showing intense fluorescence in the central vacuole following release of the vesicle contents.

immediately after mixing and at 15 and 25 h after mixing. Incubation was in the dark at 25 °C. A random sample of 181 protoplasts examined immediately after mixing showed a total of 140 vesicles bound (Fig. 1, top). After 15 h, vesicles were detected in the central vacuole (Fig. 1, middle), no vesicles were detected in the medium or still adhering to the outer surface of the plasmalemma, nor was fluorescence detected in the medium. After 24 h, vesicles were no longer detected, but the vacuoles of many protoplasts were fluorescing (Fig. 1, bottom).

Further work is in progress to prepare vesicles with less stable membranes than those described here, which on fusion with the plasmalemma may release their contents directly into the cytoplasm. The present study suggests that charged vesicles may provide a method of introducing quantifiable amounts of materials into plant protoplasts.

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Continuous production of monoclonal mouse IgE antibodies with known allergenic specificity by a hybrid cell line

SINCE the identification of IgE antibodies as mediators of allergic reactions by Ishizaka *et al.*¹ extensive work has been done to purify this new type of immunoglobulin from the serum of allergic patients and animals. However, all attempts so far have been limited by the very low serum concentration of these reaginic antibodies. This difficulty has been partly overcome with the detection of the extremely rarely occurring IgE-producing myelomas of the human² and the rat³, although IgE myelomas of other species are lacking. Moreover, none of the available IgE-myeloma proteins has a known allergenic specificity, which is indispensable for several important applications to future studies on the origin and mechanism of allergic processes. Here we describe a new murine cell line which is continuously producing high amounts of IgE antibodies with anti-ovalbumin (OA) specificity. This cell line was generated by cell fusion of murine myeloma cells with spleen cells from OA-hyperimmunised mice following the cell hybridisation technique developed by Köhler and Milstein⁴.

Table 1 PCA reaction of serum and ascites from IgE-14-205 tumour-bearing mice

Route of application	Time after inoculation (d)	Tumour rate	PCA titre	
			Serum	Ascites
Subcutaneous	8	5/5	8	
	18	5/5	1,024	
Intraperitoneal	8	4/5	32	
	18	6/6	50,000	131,000
Intravenous	8	0/3	2	
	22	2/3	3,000	

Female BALB/c mice were inoculated with 5×10^5 hybrid cells from *in vitro* culture. log 2 dilutions of serum and ascites, 0.1 ml each, were injected intracutaneously at the back cover of male Wistar rats. After 48 h the animals were challenged with an intravenous injection of 0.5 ml 0.6% OA (5 times crystallised, Serva) in PBS containing 2% Evans blue. After 30 min the animals were killed. PCA titres are expressed as the reciprocal value of the highest dilution giving a PCA reaction.

The myeloma cell line P3-X63-Ag8, is an 8-azaguanine-resistant clone derived from the IgG1 (κ)-synthesising MOPC 21 myeloma of BALB/c origin. Spleen and mesenteric lymph-node cells for fusion were derived from female BALB/c mice immunised three to four times intraperitoneally at monthly intervals with 1–2 μ g of OA with Al(OH)₃ as described by Kulczycki *et al.*⁵. Three to seven days after the last immunisation spleen and mesenteric lymph-node cells were hybridised with P3-X63-Ag8 tumour cells at a ratio of 10:1 in the presence of 42% (w/v) polyethylene glycol (PEG), molecular weight 2,000 or 4,000, according to the method of Hämmerling⁶. The hybridised cells were resuspended in HAT medium (Dulbecco's modified medium supplemented with 10% heat-inactivated fetal calf serum, 5×10^{-5} M mercaptoethanol and hypoxanthine, aminopterin and thymidine⁷) and seeded at a density of 1×10^5 to 1×10^6 cells per well (serial dilutions) into multi-well tissue culture plates (Costar 3524). Because of the hypoxanthine guanine phosphoribosyl transferase (HGPRT)-deficiency of the P3-X63-Ag8 tumour cells, this HAT medium permits only growth of hybrid cells. Supernatants of wells containing hybrid clones were tested 3–5 weeks after cell fusion for IgE anti-OA antibodies, using the passive cutaneous anaphylaxis test of the rat (PCA) as described by Ovary⁸.

Table 2 Specificity for OA of antibody IgE-14-205

IgE supernatant	Allergen	PCA titre
+	OA	32
+	—	0
—	OA	0
+	BSA	0
Heat inactivated (2 h, 56 °C)	OA	0

For experimental detail see Fig. 1.

More than 2,000 individual hybrid cell lines were investigated. Only one of these was found to be positive in the PCA test. This hybrid cell line was found in a culture plate with vigorous cell growth in all wells, suggesting the presence of several hybrid clones in each.

The cells of this culture were therefore recloned several times by serial dilutions in Costar plates. A single PCA-positive clone from a culture plate with cell growth in less than 30% of all wells was chosen and recloned three more times by the same procedure, resulting in 100% PCA-positive subclones. A particular subclone (IgE-14-205) was selected for further propagation in spinner cultures.

The IgE anti-OA production was continuously controlled by the PCA reaction and it has now persisted for more than 8 months. The PCA titre of cell culture supernatants ranged between 1:64 and 1:256. The hybrid clone IgE-14-205 could also be propagated *in vivo* in adult BALB/c mice after subcutaneous or intraperitoneal inoculation of 5×10^5 hybrid cells (Table 1). Large quantities of IgE anti-OA antibodies were produced *in vivo*, especially by intraperitoneally growing tumours. The PCA titres ($>100,000$, see Table 1) by far exceeded those which can be reached by hyperimmunisation of mice.

The following findings demonstrate that antibody IgE-14-205 belongs to the IgE class and behaves like a typical murine reaginic antibody. (1) After heating to 56 °C for 2–4 h, IgE-14-205 cell culture supernatants had completely lost their skin-sensitising capacity (Table 2). This finding is consistent with the well known thermolability of IgE antibodies⁹, which is in contrast to all other immunoglobulin classes. (2) Sheep anti-rat IgE antiserum (Miles) in the Ouchterlony immunodiffusion test¹⁰ gives a single precipitation band against serum and ascites of IgE-14-205 tumour-bearing mice (data not shown). Moreover, the skin-sensitising capacity of IgE-14-205 cell culture supernatants could be removed by *in vitro* preincubation with this antiserum and subsequent centrifugation (Fig. 1). (3) Antibody IgE-14-205 shows no immunological crossreactivity

Fig. 1 Specificity of antibody IgE-14-205. PCA reaction of antibody IgE-14-205 was measured after preincubation of cell culture supernatants (24 h at 4 °C) with equal parts of log 2 dilutions of a, OA (1,000 to 32 μ g ml⁻¹ PBS); b, sheep anti-rat IgE (undiluted to 1:32 in PBS); c, BSA (1,000 to 32 μ g ml⁻¹ PBS). After preincubation the samples were centrifuged for 10 min at 10,000 r.p.m. and the supernatants were tested in the PCA. The PCA reaction was quantitated by extracting the blue skin plaques and measuring the extinction at 623 nm (= PCA units) as described by Spector¹¹. Each supernatant was tested in duplicate. The control supernatant was diluted 1:2 with PBS.

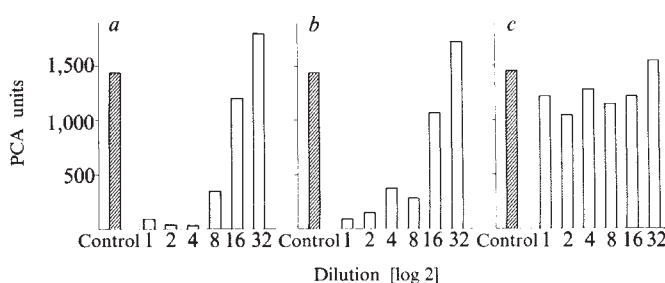


Table 3 Passive peritoneal anaphylaxis of the rat

Experimental group	No. of animals	Histamine release (IU)	(%)
Control with IgE	8	1.58 ± 0.31	100
Control without IgE	9	0.57 ± 0.05	0
5 mg per kg DSCG	5	0.74 ± 0.19	17

Male Wistar rats were injected intraperitoneally with 2 ml 1:5 diluted ascites from IgE-14-205 tumour-bearing mice (PCA titre 3,000). After 2 h 1 ml DSCG in PBS (5 mg per kg body weight) and 30 s later 5 ml OA in PBS (0, 4 mg ml⁻¹) containing 50 µg ml⁻¹ heparin were given intraperitoneally. After 5 min the animals were killed and the histamine content of the cell-free peritoneal exudate was estimated according to the method of Shore¹⁴. The animals of the negative control group received PBS instead of the IgE-ascites. IU, spectrofluorimetric intensity units.

with murine IgG2a, IgG2b, IgG3 and IgA (data not shown). It has a molecular weight of about 200,000, estimated by Sepharose-6B electrophoresis. (4) The allergenic specificity for OA of antibody IgE-14-205 was established by the demonstration that IgE antibodies from IgE-14-205 cell culture supernatants could be neutralised *in vitro* in a dose-dependent manner with OA but not with bovine serum albumin (BSA) (Fig. 1). In agreement with this finding is the observation that IgE-14-205 supernatants failed to produce a PCA reaction when either the related allergen (OA) was omitted or when an unrelated allergen such as BSA was used (Table 2). (5) When IgE-14-205 cell culture supernatants or serum and ascites from IgE-14-205 tumour-bearing mice were tested on rats, OA-specific PCA was observed. This result agrees with the well established cross reactivity of mouse and rat IgE antibodies with rat mast cells. Histologically, the PCA reaction in the rat was found to be a typical allergic reaction, with subcutaneous mast cell degranulation and dilatation of small blood vessels without any signs of a nonspecific inflammatory process. (6) Histamine release from peritoneal rat mast cells was induced in the passive peritoneal anaphylaxis test in rats¹² by serum and ascites from IgE-14-205 tumour bearing mice (Table 3). This allergic mediator release could be inhibited by anti-allergic drugs. Thus histamine release was almost completely blocked by 5 mg per kg body weight disodium cromoglycate (DSCG, Fisons) (Table 3).

In conclusion, the results described here confirm that the IgE-14-205 cell line represents a hybrid cell line which secretes OA-specific antibody of the IgE class. In addition to the reaginic antibody, the original hybrid cell line IgE-14-205 secretes the IgG1 (κ) immunoglobulin of the tumour P3-X63-Ag8, demonstrated in the Ouchterlony test with a rabbit anti-mouse IgG1 antiserum (Nordic). However, we have now selected two sublines which are highly active in secreting the reaginic antibody without any detectable IgG1 (κ) immunoglobulin in cell culture supernatants (data not shown).

Thus, the described hybrid cell line is the first tumour cell line which is continuously synthesising murine reaginic antibodies. It enables the production of murine IgE for various immunochemical, allergological and genetic studies, which have hitherto been extremely difficult¹³. Furthermore, antibody IgE-14-205 is the first available monoclonal IgE with known allergenic specificity. The murine hybrid cell line IgE-14-205 will be available on request exclusively for scientific purposes.

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Heavy HLA-DR (Ia) antigen chain is controlled by the MHC region

THE major histocompatibility complex (MHC), called the *HLA*-region in man and the *H-2* region in the mouse, contains many genes controlling an array of immunobiological phenomena. Thus certain parts of the immune response and various cell-cell interactions in normal immune processes are under genetic control from the MHC². Two structurally distinct groups of cell surface glycoproteins have so far been identified as products of genes localised to the MHC. The *HLA-A, B* and *C* loci control the expression of dimeric molecules composed of dissimilar subunits^{3,4}. The small subunit, β_2 -microglobulin, is invariant and its structural gene resides on chromosome 15⁵. The large subunit carries the alloantigenic determinants which show that the *A, B* and *C* loci most probably harbour the structural genes for these polypeptide chains. The *HLA-D* locus also controls expression of cell surface antigens which are composed of two types of dissimilar subunits, and seem to be the human counterparts to murine Ia antigens⁶⁻⁹. Neither in man nor in the mouse have unambiguous data been provided demonstrating whether only one or both *HLA-D* (Ia) antigen subunits express the particular allotypic determinants. However, Barnstable *et al.*¹⁰ approached this problem by using the somatic cell hybridisation technique and provided evidence suggesting that at least the large, 35,000 molecular weight *HLA-D* antigen subunit is controlled by the MHC. In this note we demonstrate that by serological analyses and peptide mapping experiments the 35,000 MW *HLA-D* antigen chain is a product of the *HLA-D* locus.

HLA-D antigens were isolated from leukaemia cells of a patient suffering from chronic lymphatic leukaemia, as will be described elsewhere (L.K. *et al.* in preparation). The isolated *HLA-D* antigens were of high purity as revealed by SDS-polyacrylamide gel electrophoresis, gel chromatography, ultracentrifugation and immunological analyses. The *HLA-D* antigen phenotype of the patient, from whom cells were derived, was Dw3 and Dw4 as assessed by tissue typing techniques¹¹. The highly purified *HLA-D* antigens reacted with alloantisera directed against Dw3 and Dw4 but not with an alloantiserum directed against Dw2.

To establish which of the two *HLA-D* antigen subunits reacts with the alloantisera, ¹²⁵I-labelled¹² *HLA-D*-antigens were subjected to isoelectric focusing (Fig. 1). The material resolved into three main radioactive peaks, material from the peaks was extracted with a Tris-buffer containing Triton X-100 and aliquots were subjected to SDS-polyacrylamide gel electrophoresis¹⁴. The most acidic and basic fractions comprised the two *HLA-D* antigen subunits in highly purified form. The intermediate peak (c in Fig. 1) varied in size in different experiments. The observation that the amount of this material was inversely proportional to the amounts of the acidic and basic

components together with the SDS-polyacrylamide gel electrophoretic profile strongly suggest that the intermediate material (peak *c*) represents undissociated *HLA-D* antigens.

The separated subunits and the intact, radioactively labelled *HLA-D* antigens were treated with various alloantisera and heteroantisera in indirect immunoprecipitation assays¹⁵ to elucidate their antigenic properties. Table 1 summarises the results. The rabbit antiserum against *HLA-D* antigens¹⁵ reacted well with the intact *HLA-D* antigens as well as with the isolated subunits whereas the antiserum raised against *HLA-A, B* and *C* antigens³ did not react significantly better than normal rabbit serum. The alloantisera did not react as well with the intact *HLA-D* antigens as the rabbit antiserum, but it is obvious that each alloantiserum only recognised a subpopulation of the isolated *HLA-D* antigens as the combination of alloantisera precipitated about as much *HLA-D* antigens as the sum of the individual alloantisera. Alloantiserum directed against the specificity Dw2 did not react with the *HLA-D* antigens which

Table 1 Immunological reactivity of *HLA-D* antigens and their isolated subunits

Antiserum*	<i>HLA-D</i> antigens	28,000 MW chain % precipitated	35,000 MW chain
R α <i>HLA-D</i>	90	65	54
R α <i>HLA-A, B, C</i>	2	3	2
NRS	3	1	1
Dw3	34	4	16
Dw4	24	3	10
Dw3 + Dw4	52	3	21
Dw2	3	1	2
NHS	2	2	1

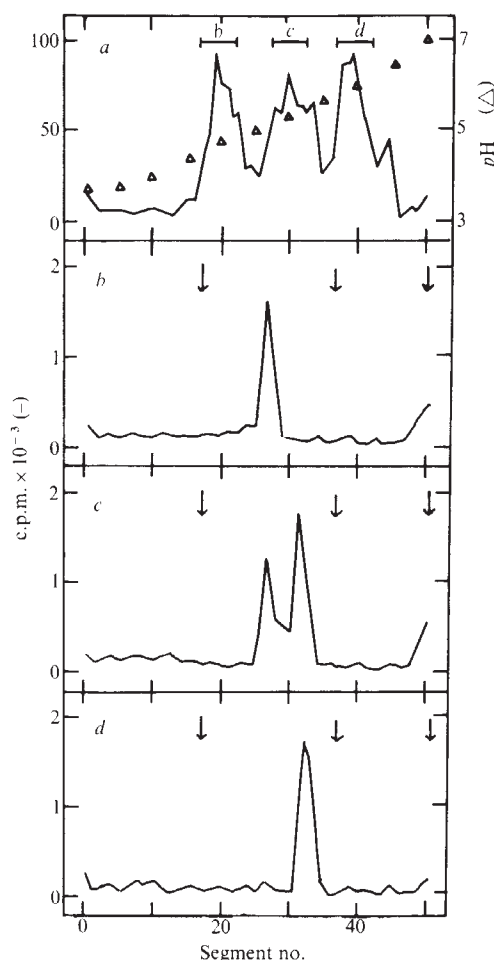
The ¹²⁵I-labelled *HLA-D* antigens and the separated subunits were incubated with 25- μ l portions of the alloantisera or 3- μ l portions of the rabbit sera as indicated above. After 5 h at room temperature immune complexes and free immunoglobulins were precipitated¹⁶. The precipitates were washed three times with ice-cold saline and subjected to SDS-polyacrylamide gel electrophoresis to establish subunit composition. The radioactivity in the precipitated proteins was measured. The data represent a typical experiment. Variations in the amount of radioactivity precipitated with the various antisera were noted in different experiments, although the data were qualitatively identical. Preliminary analyses suggest that the immune reactivity of the *HLA-D* antigens and the isolated subunits depends on the iodination conditions and the time spent in the urea-containing buffer.

The designations for the sera are: R α *HLA-D*, rabbit antiserum against *HLA-D* antigens; R α *HLA-A, B, C*, rabbit antiserum against *HLA-A, B, C* antigens; NRS, normal rabbit serum; Dw2, Dw3, Dw4, alloantisera against antigenic specificities Dw2, Dw3 and Dw4 respectively; NHS, normal human serum. The alloantisera were monospecific as determined by their reactivity in tissue typing experiments with use of the same target cells as used in the VII Histocompatibility Testing Workshop (Oxford). Their local designations are 3082B (Dw2), 4551 (Dw3) and 2503C (Dw4).

agrees with the results of the tissue typing (see above). Neither of the alloantisera recognised the 28,000 MW subunit whereas both antisera precipitated significant amounts of the 35,000 MW chain. The reactivity of the anti-Dw4 serum was considerably weaker than that of the anti-Dw3 serum. This observation is in part explained by the differences in titre between the two sera but the urea treatment used during the isolation procedure for the subunits may have deranged the Dw4-expressing *HLA-D* antigens more than the Dw3-expressing ones.

The lack of reactivity of the alloantisera against the 28,000 MW *HLA-D* antigen subunit may have depended on this chain being more sensitive towards denaturants than the 35,000 MW chain. This possibility had to be considered although the rabbit antiserum reacted well with the isolated 28,000 MW chain in as much as alloantigenic determinants may reside in limited parts of the chain. Further attempts to document an allotypic variation also for the 28,000 MW subunit were therefore made. ³H-Tyrosine-labelled *HLA-D* antigens were isolated by indirect immunoprecipitation¹⁶ using rabbit anti-*HLA-D* antigen serum. The sources of *HLA-D* antigens were the lymphoblastoid cell lines Daudi and Raji, which display the *HLA-D* phenotype Dw6/unidentified and Dw3/Dw6 respectively (C. Barnstable, personal communication). The *HLA-D* antigen subunits were separated by SDS-polyacrylamide gel electrophoresis. By running the electrophoresis in the absence of reducing agents a complete separation between the two subunits was obtained (compare ref. 7). The two types of subunits were separately extracted from the gel, digested with trypsin and subjected to peptide separation by high-pressure liquid chromatography. The sensitivity and reproducibility of this method makes it well suitable for the fractionation of radiochemical amounts of peptides and it has recently been used effectively for the isolation of peptides derived from trace amounts of *HLA-A* and *B* antigens (L.R., J.F. and P.A.P., in preparation). Figure 2 shows the results of the peptide separations. In analogy with the serological analyses described above

Fig. 1 Isoelectric focusing of highly purified ¹²⁵I-labelled *HLA-D* antigens. The labelled antigens were treated with 9 M urea in a buffer containing 2% Ampholines 3.5–10 (LKB) and 2% Triton X-100 for 2 h. The material was then subjected to isoelectric focusing¹³ in a 6% polyacrylamide gel containing 1%, pH 3.5–5, 1%, pH 5–8, and 0.5%, pH 3.5–10. Ampholines, 0.3 M sucrose, and 2% Triton X-100. After completed separation the gel was sectioned in an automatic gel slicer. Fractions containing radioactivity were pooled as indicated in the figure (*b–d*) mixed with 0.2 ml of 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 0.5% Triton X-100, and gently agitated for 12 h to extract the radioactive material. Polyacrylamide was then removed by filtration over glass wool, and the radioactive materials were subjected to dialysis against the Triton X-100-containing Tris buffer. Aliquots from the three fractions were subsequently subjected to SDS-polyacrylamide gel electrophoresis¹⁴ (*b–d*). The arrows denote the migration positions of marker immunoglobulin G heavy and light chains and the tracking dye.



the 35,000 MW subunits derived from the Daudi and Raji cells displayed clearly discernible peptide differences. However, the peptide patterns recorded for the 28,000 MW chains were very similar. As can be seen in Fig. 2 the two types of *HLA-D* antigens subunits from a single cell line did not exhibit peptide patterns that argue for any obvious identity in primary structure. Since the procedure for the peptide mapping experiments was very reproducible it seems reasonable to conclude that the Daudi and Raji cell lines express very similar if not identical 28,000 MW *HLA-D* antigen subunits, whereas the 35,000 MW chains have different primary structures.

The data presented here, together with those of Barnstable *et al.*¹⁰, seem to establish that the large *HLA-D* antigen subunit by serological, structural and genetical evidence is derived from the MHC. By the same criteria there is no evidence to suggest that the small *HLA-D* antigen chain is coded for by the same genetic region. However, the I-region of the mouse controls the expression of *HLA-D* antigen analogues (the Ia antigens) from at least two loci, A and E/C¹⁷. Limited amino acid sequence analyses

have demonstrated that both Ia-antigen subunits may be derived from the MHC^{18,19}. Further work is therefore needed to clearly demonstrate that the small *HLA-D* antigen subunit is not a product of the *HLA-D* locus.

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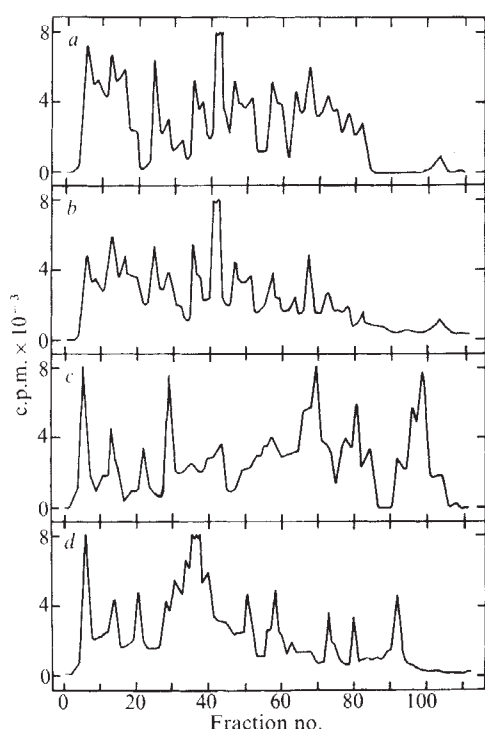


Fig. 2 Peptide patterns of ³H-tyrosine labelled *HLA-D* antigen subunits derived from Daudi and Raji cells. The labelled cells were washed, treated with 1% Triton X-100 in 0.02 M Tris-HCl buffer, pH 8.0, and the glycoproteins were isolated by affinity chromatography¹⁶. *HLA-D* antigens were isolated by indirect immunoprecipitation and immune complexes were separated by SDS-polyacrylamide gel electrophoresis¹⁴ in the absence of reducing agents. After segmentation of the gels the well-resolved *HLA-D* antigen subunits were extracted from the gel pieces essentially as described in Fig. 1. After the extraction remaining polyacrylamide gel was removed by filtration over glass wool. Human IgG (100 µg) was then added to each pool which was subjected to extensive reduction and alkylation. Excess reagents and SDS was removed by gel filtration on columns of Sephadex G-25 equilibrated with 0.02 M NH₄HCO₃. After lyophilisation the samples were taken up in 0.4 ml of 0.2 M NH₄HCO₃ containing 10 µg of trypsin. The digestions were terminated by lyophilisation after 3 h at 37 °C. The tryptic digests were finally dissolved in 50 µl of 0.05 M ammonium acetate, pH 4.5, containing 6% acetonitrile and subjected to high pressure liquid chromatography (Waters Associates). The C-18 column was operated at 50 °C. Elution was performed with a 60-ml linear gradient of acetonitrile, 6% to 40%, in the ammonium acetate buffer. Fractions of 0.3 ml were collected at 20 s intervals. a, Daudi 28,000 MW chain; b, Raji 28,000 MW chain; c, Daudi 35,000 MW chain; d, Raji 35,000 MW chain.

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9,11-Epoxyiminoprostano-5,13-dienoic acid is a thromboxane A₂ antagonist in human platelets

THROMBOXANE A₂ (TXA₂), an important arachidonic acid metabolite, aggregates platelets¹⁻⁶, constricts coronary smooth muscle^{1,7,8}, and is formed during mechanical or anaphylactic organ challenge⁹⁻¹¹. There are selective thromboxane A₂ synthetase inhibitors¹²⁻¹⁶ but there are no known, selective TXA₂-receptor antagonists. We report here that 9,11-epoxyiminoprostano-5,13-dienoic acid¹⁷ (9,11-EIP) antagonises TXA₂-mediated platelet aggregation without inhibiting thromboxane synthetase or fatty acid cyclo-oxygenase. This finding should allow further exploration of the nature of TXA₂ receptors in human platelets.

The fatty acid cyclo-oxygenase enzyme transforms arachidonic acid into prostaglandin (PG) endoperoxides (PGG₂, PGH₂)^{18,19}, which aggregate platelets either directly^{20,21} or after conversion to TXA₂^{1,4,22}. Platelet-rich plasma (PRP) and washed platelet suspensions containing 9,11-EIP were challenged with the following stimuli: arachidonic acid, PGH₂, TXA₂ generated *in vitro* by platelet microsomes⁴ and a sub-aggregatory dose of PGH₂, PG endoperoxide analogues^{23,24}, adrenaline or ADP. The aggregation profiles elicited by these agents were subsequently monitored. TXB₂ and PGE₂ concentrations during aggregation were quantitated by radioimmunoassay^{25,26}.

9,11-EIP suppressed TXA₂-induced aggregation of PRP in a dose-dependent manner (Fig. 1) with 50% inhibition occurring at <200 ng ml⁻¹. 9,11-EIP did not inhibit TXA₂ formation, because the TXA₂ was generated *in vitro* and added after its

formation. The results were identical in platelets treated with cyclo-oxygenase inhibitor (1×10^{-4} flurbiprofen), ruling out any contributions from the release of endogenous arachidonic acid metabolites. 9,11-EIP also antagonised aggregation induced by PG endoperoxide analogues^{23,24} (Fig. 2).

9,11-EIP suppressed PGH₂- and arachidonic acid-induced platelet aggregation in a dose-dependent manner at concentrations which did not affect TXB₂ levels (Fig. 3). PGE₂ and cyclic AMP levels were also unchanged (data not shown). Selective thromboxane synthetase inhibitors divert PGH₂ metabolism towards the formation of the bis-enoic prostaglandins, some of which, for example, PGD₂, can oppose aggregation by elevating cyclic AMP²⁸. Therefore, 9,11-EIP blocked aggregation independently of two common anti-aggregatory mechanisms, TXA₂ synthetase inhibition and cyclic AMP elevation²⁸. Results were similar in PRP. 9,11-EIP suppressed arachidonic acid- or PGH₂-induced aggregation in a dose-dependent manner at concentrations which did not affect TXB₂, PGE₂ or cyclic AMP levels. 9,11-EIP did not affect the first wave, but it suppressed the second wave of adrenaline- and ADP-induced aggregations, revealing its specific involvement with the arachidonic acid

Fig. 1 Effect of 9,11-EIP on TXA₂-induced aggregation of human PRP. PRP was prepared by centrifuging fresh, human whole blood (1 part 3.8% citrate/9 parts blood) at 200g for 10 min at 25 °C. TXA₂ was generated by incubating a sub-aggregatory concentration of PGH₂ (200 ng) with flurbiprofen-treated human platelet microsomes⁴ suspended in Tris buffer, pH 8.0 (20 µl containing 1 mg protein), for 20 s at 37 °C inside an aggregometer cuvette. PRP, preincubated at 37 °C for 2 min with 9,11-EIP was added to the cuvette containing TXA₂. The PRP was stirred at 1,100 r.p.m. at 37 °C and the aggregation profile was monitored with a Payton single-channel aggregometer. Increasing concentrations of 9,11-EIP suppressed TXA₂-induced aggregation in a concentration-dependent fashion. Effects from TXA₂ synthetase inhibition were precluded, as TXA₂ was generated *in vitro* before the addition of PRP and results were identical with PRP treated with 10^{-4} M cyclo-oxygenase inhibitors (aspirin, flurbiprofen). Results are consistent with TXA₂ antagonism. a, Control; b, 125 ng ml⁻¹; c, 250 ng ml⁻¹; d, 500 ng ml⁻¹; e, 2,500 ng ml⁻¹ 9,11-EIP.

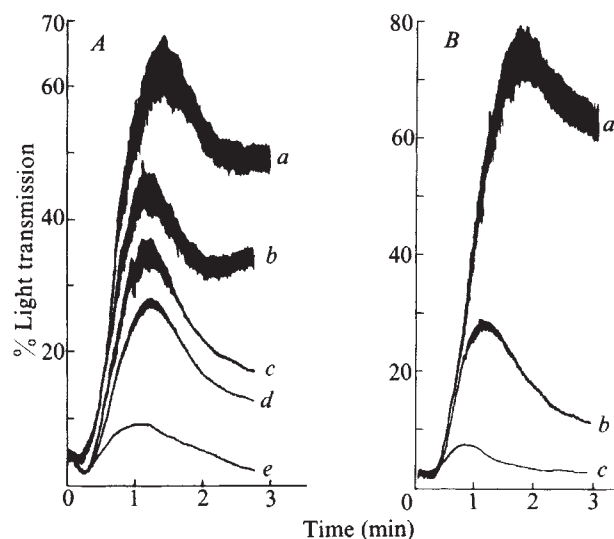
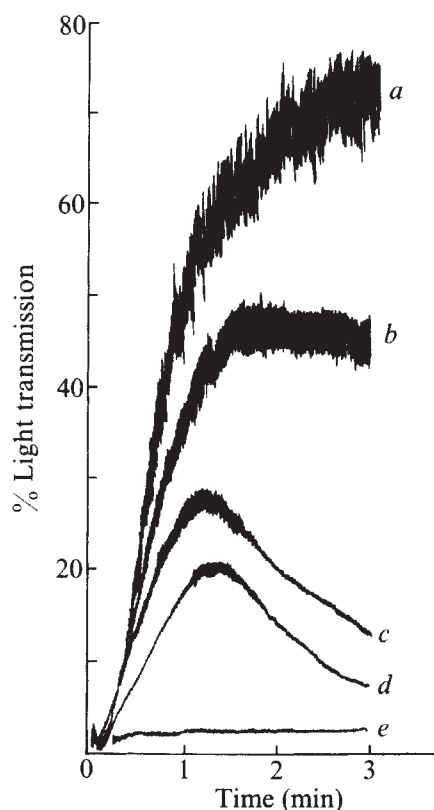


Fig. 2 Effect of 9,11-EIP on endoperoxide analogue-induced aggregation of human PRP. The conditions were similar to those of Fig. 1 except the endoperoxide analogues, 9,11-azo-15(S)-hydroxyprosta-5,13-dienoic acid (50 ng ml⁻¹) (A) or 9,11-methanoepoxy-15(S)-hydroxyprosta-5,13-dienoic acid (250 ng ml⁻¹) (B), were used so as to induce aggregation instead of TXA₂. The quantitatively different response of the antagonist against these analogues may reflect the fact that 9,11-azo-15(S)-hydroxyprosta-5,13-dienoic acid 'resembles' TXA₂ more than does 9,11-methanoepoxy-15(S)-hydroxyprosta-5,13-dienoic acid. A: a, control; b, 50 ng ml⁻¹; c, 100 ng ml⁻¹; d, 150 ng ml⁻¹; e, 300 ng ml⁻¹ 9,11-EIP. B: a, control; b, 250 ng ml⁻¹; c, 1.0 µg ml⁻¹ 9,11-EIP.

pathway. At concentrations exceeding 5×10^{-5} to 1×10^{-4} M, 9,11-EIP inhibited the platelet cyclo-oxygenase, reducing both TXB₂ and PGE₂ synthesis.

Needleman *et al.*²⁰ proposed that thromboxanes have at least two unique but dissociated properties, rabbit aorta constriction and platelet aggregation. The results with 9,11-EIP conditionally support this proposal. Although 9,11-EIP antagonises platelet aggregation, apparently by direct TXA₂-receptor blockade, it has intrinsic agonist properties for coronary smooth muscle, constricting the rat aorta and thereby preventing an assessment of any accompanying TXA₂ antagonism in this system. 9,11-EIP also inhibits PGI₂ formation in rabbit lung microsomes and BALB 3T3 cells at 10^{-5} M. Nevertheless, the thromboxane pathway dominates arachidonic acid metabolism in human platelets. TXA₂-receptor antagonists should further clarify the role of TXA₂ in platelet aggregation.

Conclusions on the role and mechanism of action of TXA₂ are frequently drawn by comparing the effects of selective inhibitors on the synthesis of arachidonic acid metabolites, modulation of cyclic AMP metabolism, and the attendant TXA₂-mediated physiology. As these inhibitors are only relatively selective³⁰, the conclusions are tentative and subject to re-evaluation. A TXA₂ antagonist is vital for further elucidation of the biochemical, pharmacological and physiological aspects of the arachidonic acid cascade in human platelets. 9,11-EIP is such an antagonist, with effects distinct from TXA₂ synthetase inhibition. Just as experiments with dichloroisoproterenol³¹ advanced the understanding of the β -adrenergic receptor, we believe that experiments with 9,11-EIP will advance the understanding of the TXA₂ receptor in platelets.

In summary, 9,11-EIP inhibited TXA₂-induced platelet aggregation in a dose-dependent manner. This inhibition was not due to an effect on TXA₂ synthetase because TXA₂ was generated *in vitro* before addition of PRP. 9,11-EIP also suppressed arachidonic acid- and PGH₂-induced aggregations of washed platelet suspensions and PRP without altering TXB₂,

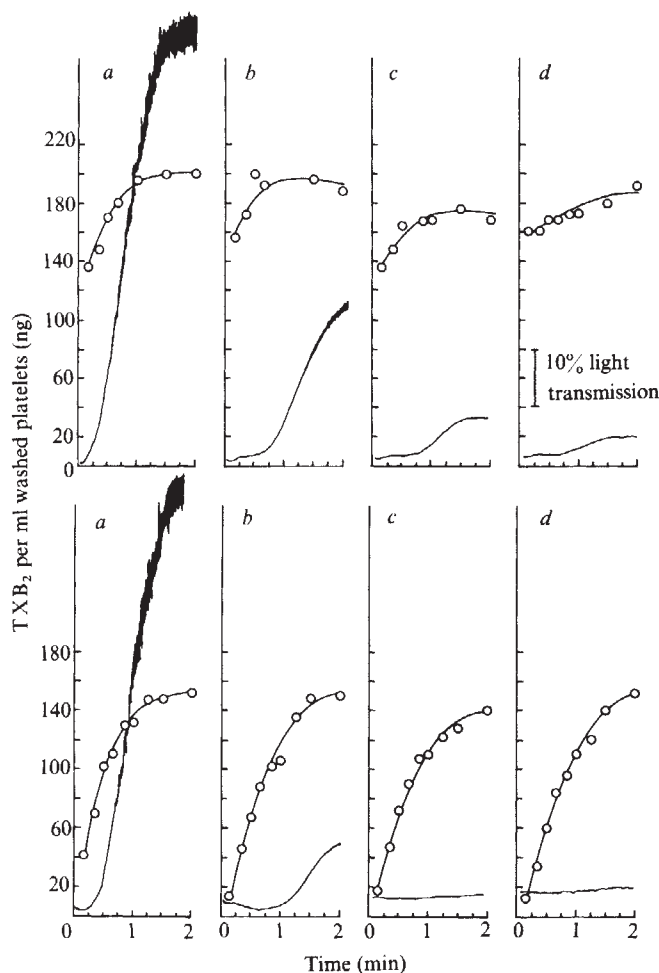


Fig. 3 Washed platelets were prepared according to Miller *et al.*²⁷. PGH₂ was prepared according to Gorman *et al.*²⁹. Platelet suspensions were incubated with 9,11-EIP for 2 min at 37 °C. The platelet suspension was then transferred to an aggregometer cuvette containing PGH₂ (0.20 µg) (upper graphs) or arachidonic acid (1 µg) (lower graphs). The suspension was stirred at 1,100 r.p.m. at 37 °C; aggregation profiles were monitored and samples (0.1 ml) were withdrawn for the radioimmunoassay of TXB₂²⁵. 9,11-EIP suppressed aggregation in a dose-dependent fashion without inhibiting TXA₂ or PGE₂ formation. The open circles represent TXB₂ levels. PGE₂ levels (data not shown) ranged over 0.10–0.50 ng ml⁻¹. Upper graphs, a, control; b, 0.40 µg ml⁻¹; c, 0.60 µg ml⁻¹; d, 1.0 µg ml⁻¹ 9,11-EIP. Lower graphs, a, control; b, 0.08 µg ml⁻¹; c, 0.80 µg ml⁻¹; d, 2.0 µg ml⁻¹ 9,11-EIP.

PGE₂ or cyclic AMP levels. These results are consistent with antagonism of TXA₂ in platelets at the receptor level. Note that others have discovered selective prostaglandin antagonists^{32–34}.

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Saccadic eye movements and visual stability

WE rarely confuse motions of our retinal images that result from movements of objects with those that result from eye movements. A predominant explanation of the perceptual stability that survives saccadic eye movements is that visual information about image movement is weighed against an internal ('extraretinal') signal about eye movement, and that a mismatch is generally perceived as movement of the world¹. Accurate information about eye position, even during a saccade, seems to be available to the oculomotor system^{2,3}, but it is not clear that this information finds its way to mechanisms underlying perceptual stability. The experiments we describe here show that errors in localisation following saccades are matched closely by errors in the size of saccades. We infer from this that the perceptual system does not monitor the extent of a saccade but merely assumes that an intended eye movement was made correctly.

The observer sits in a completely dark room with his head held steady by a dental impression. Ahead of him is an oscilloscope with an aluminised screen (P31 phosphor) on which appears a sharp, bright fixation spot (more than 2 log units above foveal threshold and diameter 2–4 arc min, depending on viewing distance), with no surrounding glow. After 2.4 s this spot moves instantaneously a fixed distance to the left or right, where it remains for 100 ms before being extinguished. The direction of step is unpredictable to the observer, who tries to re-fixate the target, but this is extinguished before an eye movement can begin⁴ and so the saccade is made in the dark. 500 ms after extinction of the target, and after the saccade has been completed, a 'probe' spot appears for 500 ms at one of several fixed points about the target position, and the observer indicates with a keyswitch whether that spot lies to the left or to the right of the target.

This cycle is controlled by a computer and is repeated many times, with the direction of the target step and the position at which the probe spot appears, varying randomly from trial to trial. In a normal experimental session 350 trials are run in which the target steps to the right or left with approximately equal frequency, and for each direction of step the probe may appear in one of seven positions around the target position. From this we are able to plot two graphs, one for leftwards displacements, the other for rightwards, showing the relative frequency with which the probe in its various positions appeared to the left or right of the target. One such pair of graphs is shown in Fig. 1, for a target that stepped either 3.3° to the left (filled circles) or right (open circles). The smooth lines drawn through the points are the maximum likelihood estimates of the probit regression lines⁵

(the fits of which were rejected at the 10% level of significance in only three cases out of 21) from which one can obtain estimates of the mean and standard deviation of the distribution of judgements. The standard deviation of judgements is about 12 arc min, and the constant error, also about 12 arc min, is such that after a saccade the peripheral target seems to have moved further away from the fixation spot.

These errors, although small, are very reliable and indicate that in an impoverished environment the observer is not fully able to compensate for image displacements that result from saccades. The constant errors described here are a little smaller than those found by Monahan⁶ and Matin⁷ and much smaller than errors found by Bridgeman *et al.*⁸ in experiments to investigate perceptual mislocations related to saccadic eye movements; the task of subjects in the latter experiment was quite unlike that used here, which may explain the disagreement.

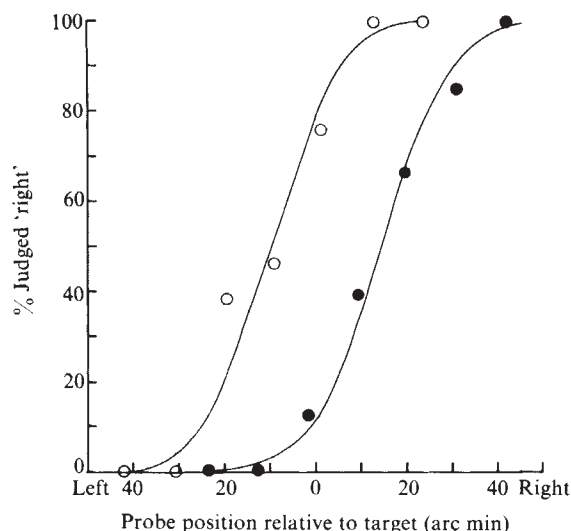


Fig. 1 Frequency with which the probe spot was judged to lie to the right of the target position (0 on the abscissa). The abscissa indicates probe position relative to the target. ● judgements made when a saccade followed a step of 3.3° to the left; ○, corresponding judgements made following a step to the right. Smooth curves are maximum likelihood estimates of the probit regression lines. Each curve is based on an average of 175 trials in a single session, during which the probe appeared randomly at one of seven positions around the target point (observer P.L.).

Constant errors might have arisen because the initial target step was underestimated, although the saccades were made accurately to the (mislocated) target, or the saccade to the correctly located target may have been supposed accurate when in fact it fell short. Variability is probably due principally to two sources: first, the target is seen (initially) in peripheral vision, so its visual direction is to some extent uncertain. Moreover, the observer has to remember this direction during the 500 ms between target disappearance and the reappearance of a spot. Second, the extent of the eye movement to this uncertain, poorly remembered target may not be properly registered. The combined influence of these factors produces only small errors in localisation, a finding that might be interpreted as evidence for a reliable extraretinal signal that specifies the extent of the saccade, but an analysis of eye movements made during the experiment suggests otherwise. The horizontal position of the eyes was monitored (with a resolution of ± 4 arc min) by measuring the amount of infrared light reflected from slit images that lay horizontally over the corneo-scleral boundary, and a continuous record of eye position, together with a record of target position, was made on an oscillograph.

These measurements, a sample of which is shown in Fig. 2, indicate that at the time the probe appears the eye has stopped

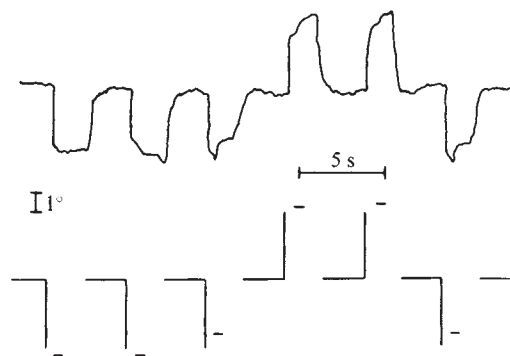


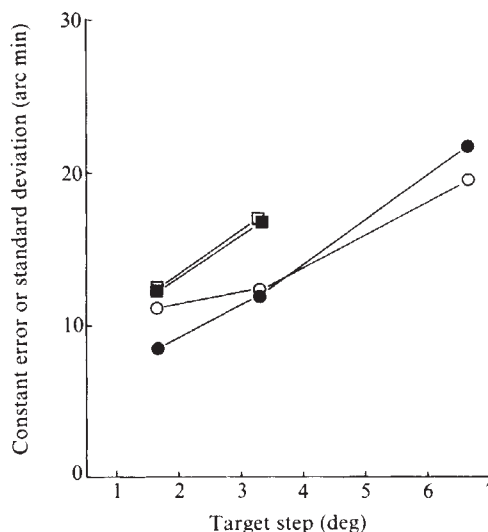
Fig. 2 Sample record of eye position during an experiment. The target stepped randomly 3.3° to the right or left and after 100 ms was extinguished. 500 ms later a probe appeared randomly at one of seven positions about the target point. The observer had to indicate whether the probe appeared to the right or left of the target. Corrective saccades occurred frequently after the probe appeared, usually so as to reduce an undershoot. Blank portions of the stimulus trace indicate that no spot was visible.

consistently short of the target by about 17 arc min, an amount close to that by which the target is mislocated. Moreover, the standard deviation of eye position (without allowance for error of measurement) is only a little greater (17 as opposed to 12.4 arc min) than that of judgements about target position.

Figure 3 shows that for steps in target position of 1.15° the mislocation is also close to the error in eye movement. Accurate measurement of eye movements to target steps of 6.6° was not possible, but errors in localisation follow the trend for smaller saccades.

Our results show that, following a saccade, the spot judged to lie in the target position is the one that falls on the fovea. Thus, to explain the small errors in locating targets after a saccade, we need only suppose that the observer relies on the eye movement being accurate and locates at the target position the spot that falls on the fovea. It is unnecessary to postulate an extraretinal signal that specifies accurately the size of the saccade; clearly,

Fig. 3 Accuracy and reliability of judgements about target location following saccades to target steps of different sizes (circles). Both parameters were estimated from probit regression lines. Points are averages for target steps to left and right, and a positive constant error indicates that the probe judged to lie on target was too close to the original fixation point. Accuracy and reliability of saccades to target steps of different sizes (squares). Points are averages for saccades to left and right. Open symbols, standard deviation; filled symbols, constant error.



some signal is needed to indicate that the eye has moved, but more precise information is not required.

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Vanadate-stimulated natriuresis

THE observation^{1–3} that V(v) is a potent inhibitor of (Na⁺, K⁺)-activated renal ATPase raised the possibility that it has natriuretic and thus diuretic properties in the living animal. We report here experiments showing that it has.

Plastic catheters were placed in the urinary bladder and femoral vein of rats (250–350 g) under ether anaesthesia. While recovering from the anaesthetic, the animal was confined in a restraining box and placed on a laboratory balance. The opposite arm of the balance actuated the delivery of isotonic saline (145 mM NaCl, 5 mM KCl) from a peristaltic pump to the femoral vein, in such a way that, during the experiment, the urinary and evaporative losses were made good and the weight of the animal was maintained.

During the succeeding 2 h the rate of flow of urine became roughly constant. Sodium orthovanadate (1.5 µmol) was then injected intravenously in 0.15–0.3 ml of the same isotonic saline; these volumes are too small to affect urine flow by augmenting the plasma volume. Table 1, which summarises the results of four consecutive experiments, shows the total excretion of water, sodium and potassium during the hours immediately preceding and immediately following the administration of vanadate. The flow of urine increased dramatically within a few minutes of the vanadate injection, reaching peak rates of about 1 ml min⁻¹. As this represents approximately 50% of the glomerular filtration rate⁴, it is likely that vanadate acts, at least in part, on the proximal tubule. The sodium

Table 2 Distribution of ⁴⁸V 75 min after the intravenous injection of 1.5 µmol ⁴⁸V-vanadate

Tissue	Blood plasma	Renal cortex	Renal outer medulla	Renal papilla	Urine
⁴⁸ V content (µmol per kg wet weight)	14.9	62.0	10.2	7.8	2.6

concentration in the urine produced in response to vanadate approximated to that in plasma. The integrity of the nephrons was not lost, for neither glucose nor protein was detectable in any of the samples of urine. Also, the effect of vanadate seems to be reversible, as, in longer-term experiments, urine flow following a vanadate injection diminished after a few hours, but the kidney was still capable of responding to a further dose as it had before.

In the first of the experiments included in Table 1, we used ⁴⁸V-labelled vanadate and determined the distribution of tracer in the kidney and elsewhere 75 min after its administration, when the diuresis was still nearly maximal. Table 2 shows that ⁴⁸V was selectively concentrated in the renal cortex. Otherwise, the distribution of radioactivity in the various organs was similar to that described by Hathcock *et al.*⁵. Within 75 min of the ⁴⁸V injection, 43% of the radioactivity had been excreted.

We conclude that vanadate is a potent natriuretic and diuretic substance, perhaps the most potent now known. It remains to be determined (1) whether vanadate, at the naturally occurring blood or tissue levels, has a physiological role in controlling natriuresis, and (2) whether the natriuretic and diuretic effects of vanadate can be therapeutically useful.

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Table 1 Excretion of water, sodium and potassium before and after the intravenous injection of sodium orthovanadate

Experiment	Excretion before injection			Excretion after injection		
	Water (ml h ⁻¹)	Na (mEq h ⁻¹)	K (mEq h ⁻¹)	Water* (ml h ⁻¹)	Na (mEq h ⁻¹)	K (mEq h ⁻¹)
1	5.22	0.598	0.325	49.6	7.05	0.442
2	1.53	0.212	0.267	38.7	6.10	0.333
3	1.43	0.175	0.127	27.9	2.53	0.313
4	2.10	0.220	0.197	28.5	3.91	0.301
Mean ± s.e.m.	2.57 ± 0.90	0.01 ± 0.099	0.299 ± 0.043	36.2 ± 5.1	4.84 ± 1.07	0.347 ± 0.032

The dose, 1.5 µmol, was chosen arbitrarily as that which, if it were distributed uniformly in the body water, would give a concentration sufficient *in vitro* to cause 75% inhibition of (Na⁺, K⁺)ATPase prepared from pig kidney. 1.5 µmol vanadate in a 300 g animal represents about 2.5% of the LD₇₀, determined in mice⁶; estimates of LD₁₀₀ suggest that vanadate is about equally toxic to rats and mice⁶.

* For comparison, it is worth noting that an equimolar dose of the therapeutic diuretic furosemide injected intravenously into a 300 g rat caused a diuresis averaging about 10 ml h⁻¹ during the first hour, with peak flow rates up to about 0.3 ml min⁻¹.

Peptidyl-transfer RNA hydrolase prevents inhibition of protein synthesis initiation

PEPTIDYL-tRNA HYDROLASE (called hydrolase below) catalyses the hydrolysis of peptides from peptidyl-tRNAs that are not bound to ribosomes^{1,2} but will not hydrolyse amino acyl-tRNA or fMet-tRNA^{fMet}. It is interesting that hydrolase seems to be ubiquitous in nature; it has been found in all cells where it has been sought, including yeast³, bacteria, rat liver and jackbeans⁴. However, the metabolic role of this enzyme has not been established. It is required for normal function, as a mutant strain of *Escherichia coli* with a temperature-sensitive hydrolase cannot continue protein synthesis at the nonpermissive temperature⁵. Hydrolase itself is not required for initiation, elongation or termination phases of protein synthesis⁶. Menninger⁷ has shown that peptidyl-tRNA, formed on ribosomes, accumulates at nonpermissive temperatures in cells with the temperature-sensitive hydrolase. Hydrolase thus seems to be a scavenger enzyme of peptidyl-tRNAs normally aborted from ribosomes during protein synthesis. Of interest now is why peptidyl-tRNA is frequently prematurely released from ribosomes and why protein synthesis is inhibited in the mutant strain with a defective hydrolase⁵. I report here work on the latter problem and present evidence which indicates that peptidyl-tRNAs accumulate and compete with *N*-formyl-Met tRNA^{fMet} during initiation of protein synthesis.

Two possible mechanisms of protein synthesis inhibition at 42°C in the temperature-sensitive hydrolase strain are that one or more infrequently occurring tRNAs may become sequestered as peptidyl-tRNA, resulting in inhibition of polypeptide elongation or, on the other hand, that the only known product of the reaction (peptidyl-tRNA) may interfere with initiation of polypeptides. Peptidyl-tRNA is not unlike *N*-formyl-Met-tRNA^{fMet}. Thus, these two similar compounds may compete at initiation for the P-site on the ribosome.

When the hydrolase mutant strain is incubated at temperatures intermediate between permissive growth and nonper-

missive growth (40, 40.5, 41 °C), partial inhibition of growth is observed. This is interpreted as limited inactivation of hydrolase, resulting in a reduced rate of peptidyl-tRNA hydrolysis. If elongation rates are affected at these intermediate temperatures, protein polymerisation time should be increased. If initiation of protein synthesis is inhibited, however, the polymerisation rate will remain unchanged for a specific protein.

The time lag between the induction of the lactose operon and the first appearance of β -galactosidase activity above basal enzyme level was used to estimate the peptide chain elongation rate^{8,9}. It has been shown that this approach is not a reflection of changed mRNA synthesis times⁸. Hence a measure of polymerisation time of β -galactosidase can differentiate between the two possibilities outlined above.

The data presented in Table 1 indicate that the rate of peptide-chain elongation does not change significantly with increase in growth temperature in the hydrolase temperature-sensitive strain. The chain elongation rate is slightly less in the mutant strain at 40 °C than in the parent strain (20%), but nearly identical at the permissive temperature (30 °C). However, the growth rate of the mutant strain decreases considerably as the growth temperature is increased from 37 to 41.5 °C, reflecting inhibition of protein synthesis by steadily increasing concentrations of peptidyl-tRNA in the cell. Furthermore, the relative rate of polypeptide chain initiation is proportionally decreased (Table 1, column 5). These data are derived from the initial slope of the induction curve; they reflect the relative number of molecules synthesised per unit time. The relative number of chain initiations in strain AA-7852 (*pth*^{ts}) decreases by slightly more than half from 40 to 41.5 °C, and the doubling time decreases proportionally. No significant growth or β -galactosidase induction is observed at 42°C, reflecting complete inactivation of the hydrolase.

Other data suggesting that initiation of protein synthesis is inhibited in the hydrolase mutant strain at the nonpermissive temperature come from studies of the polysome distribution after shift to 43 °C. Menninger *et al.*⁶ found that polysomes slowly dissociated at 43 °C, forming 70S subunits. Elongation continues temporarily, resulting in dissociation of polysome structures. However, initiation only forms 70S units, possibly because of a peptidyl-tRNA in the P-site. This is consistent with the idea that some poison accumulates, very probably peptidyl-tRNA, but must reach a critical level before inhibition is seen. Protein synthesis is not inhibited in the hydrolase mutant until about 10 min after transfer to the nonpermissive temperature.

In conclusion these data show that the number of polypeptide chain initiations decreases as hydrolase is partly inactivated. On the other hand, the chain elongation rate is not affected. These findings imply that hydrolase plays an important part in cell metabolism by destroying spontaneously formed peptidyl-tRNAs which, in turn, inhibit protein synthesis initiation, probably by competing with *N*-formyl-Met-tRNA^{fMet} in the P-site of the ribosome.

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Table 1 Amino acid polymerisation and growth rates at various temperatures

Strain	Growth temperature	Doubling time (min)	Polymerisation rate (amino acids s ⁻¹)	Initial slope of induction curve (nmol ONPG hydrolysed per min per 3 × 10 ⁸ cells)
AA-7852	30.0 ± 0.05	190 ± 5	5.6 ± 0.3	55
	37.0 ± 0.05	170 ± 5	6.8 ± 0.2	57
	40.0 ± 0.05	210 ± 8	7.5 ± 0.2	42
	41.0 ± 0.05	270 ± 10	7.3 ± 0.2	29
	41.5 ± 0.05	300 ± 10	7.3 ± 10	21
CP-78	30.0 ± 0.05	150 ± 5	6.1 ± 0.3	72
	40.0 ± 0.05	110 ± 5	9.1 ± 0.2	75

Cells were grown in M-9 medium with 0.4% glycerol and required amino acids in a gyrotary shaking water bath at the specified temperature⁶. Strains CP-78 (a K12 strain of *E. coli*, *arg*, *his*, *leu*, *thr*, *thi*) and AA-7852 (a single-step mutant of strain CP-78 that is now temperature-sensitive for peptidyl-tRNA hydrolase (ref. 5) were induced for β -galactosidase synthesis by addition of 5 mM 3', 5'-cyclic AMP and 1 mM isopropylthiogalactoside after growth at the specified temperature for 2 hours. The cultures were sampled every 5 s by taking 0.5 ml into test tubes containing 0.50 ml of a solution (1 mg ml⁻¹) of chloramphenicol in distilled water⁸. These samples were assayed for β -galactosidase activity by cell lysis with toluene and hydrolysis of *o*-nitrophenol- β -D-galactopyranoside (ONPG) at 25 °C. Polymerisation rate was established by the time required for the first newly synthesised β -galactosidase molecules to appear^{8,9}. There are 1,021 amino acids in β -galactosidase¹⁰. A change of 0.004 A₄₂₀ units equals 1 nmol of ONPG¹¹.

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Inhibition of Q β RNA 70S ribosome initiation complex formation by an oligonucleotide complementary to the 3' terminal region of *E. coli* 16S ribosomal RNA

It has been proposed that a key event in the recognition of a prokaryotic protein initiation site by the ribosome is the interaction between the 3'-terminal sequence of the 16S rRNA and a purine-rich sequence preceding the initiator triplet by three to nine nucleotides^{1,2}. This hypothesis predicts that if the 3'-terminal region of the 16S rRNA were blocked, binding of the ribosome to the initiator region of an mRNA should be diminished or prevented, while binding to the trinucleotide A-U-G should not be affected. We have shown that the oligonucleotide A-G-A-G-G-A-G-G-U_{OH} (P-2a), eight bases of which are complementary to the sequence A-C-C-U-C-C-U-U-A_{OH} at the 3'-terminal region of 16S rRNA ($\Delta G = -16.9 \text{ kcal mol}^{-1}$ calculated as described in Table 1), binds tightly to the 30S ribosome and is released as a complex with the 3'-terminal 49-mer of 16S rRNA after digestion with cloacin DF13 (ref. 3). We report here that this oligonucleotide either strongly inhibits Q β RNA binding to 70S ribosomes or

decreases the stability of the initiation complex formed in its presence. As we found that the formation of complexes of pA-U-G and 70S ribosomes was not inhibited by the oligonucleotide, we conclude that inhibition (or destabilisation) of Q β RNA binding is not due to an effect on the interaction between fMet-tRNA and the initiation triplet or on the association of the 30S and 50S ribosome subunits.

Incubation of the low-salt-washed ribosomes (which retain initiation factors) and intact Q β RNA in the presence of fMet-tRNA leads to the formation of a complex sedimenting at 70S (ref. 4). As in the case of all phage RNAs, ribosomes attach predominantly to the initiation site of the coat cistron, and to a lesser extent to that of the replicase cistron⁴⁻⁷.

Table 1 and Fig. 1 show the effect of preincubating ribosomes (5 pmol) with different amounts of purified P-2a before the addition of ³H-labelled Q β RNA (2 pmol). It is seen that the amount of Q β [³H]RNA in the 70S peak is about 0.4 pmol (20% of the input) in the absence of P-2a (Fig. 1, left figure) and decreases with increasing amounts of P-2a. At a molar ratio of P-2a to Q β RNA of 2.5, 50% inhibition of 70S complex formation was observed, and at a ratio of 10, inhibition was 87% (Fig. 1, right figure, Table 1). The effect of several other oligonucleotides on 70S complex formation was compared to that of P-2a in the experiment in Table 1. An equimolar mixture of P-2a and P-2b (which are difficult to separate) had the same inhibitory effect as pure P-2a.

To show that inhibition of complex formation was not due to an inhibitor other than an oligonucleotide, an aliquot of the mixture of (weakly ³²P-labelled) P-2a and P-2b was treated with Sepharose-linked RNase T₁; the equivalent of 20 pmol of the ³²P-labelled oligonucleotides, which after this treatment were completely digested (as shown by electrophoretic analysis) gave virtually no inhibition (Table 1). The RNase A-resistant oligonucleotides G-G-A-A-G-G-A-G-C_{OH} (P-4) and G-G-A-G-A-A-G-C_{OH} (P-6), which showed less complementarity with the 3'-terminal region of 16S rRNA (Table 1) than P-2a, were far weaker inhibitors; the concentrations required to give 50% inhibition were about three and five times greater, respectively,

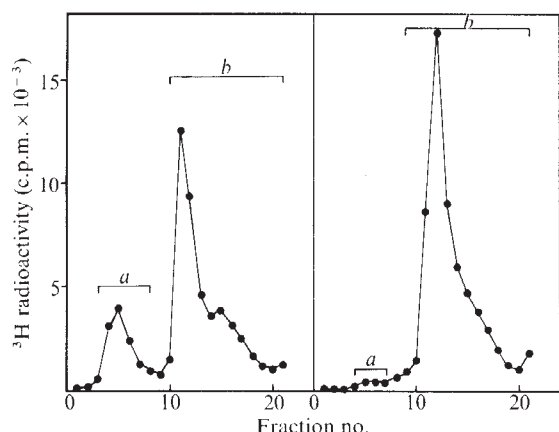


Fig. 1 Effect of oligonucleotide P-2a on binding of Q β [³H]RNA to 70S ribosomes. The preparation of the pancreatic oligonucleotide P-2a will be described elsewhere³. Q β [³H]RNA, fMet-tRNA and ribosomes were prepared as described previously¹⁴. Low-salt-washed ribosomes (5 pmol) were incubated with or without P-2a (20 pmol) in 50 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 120 mM NH₄Cl, 0.5 mM GTP, 0.5 mM dithiothreitol and about 440 pmol of unfractionated, stripped tRNA charged with fMet (ref. 14) (estimated to contain 10 pmol of fMet-tRNA) in a volume of 10 μ l for 15 min at 37 °C. Then, 2 pmol of Q β [³H]RNA (specific activity, 3×10^5 c.p.m. pmol⁻¹; in 2 μ l) were added to the reaction mixture and incubation was continued for 10 min at 37 °C. 100 μ l of buffer B (10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NH₄Cl and 10 mM β -mercaptoethanol) were added and the sample was loaded on a 4 ml 10–30% sucrose gradient in buffer B. Centrifugation was for 90 min at 58,000 r.p.m. and 4 °C in an SW60 Spinco rotor. 21 Fractions were collected on paper disks (Schleicher and Schuell, LS78 Ruffi, diameter 22 mm) and ³H radioactivity was determined in POPPOP-scintillator. The A₂₆₀ profile of a mixture of 30S, 50S and 70S ribosomes, run in the same conditions, was used to locate the ³H radioactivity sedimenting at 70S (indicated by a). The amount of 70S ³H radioactivity was calculated relative to the total ³H radioactivity recovered from the gradient (a + b). Left hand figure, without P-2a, right, with P-2a (20 pmol).

Fig. 2 Sedimentation analysis of complex formation between ribosomes and Q β [³H]RNA and [³²P]pA-U-G: effect of fMet-tRNA and oligonucleotide P-2(a+b). The reaction mixture contained 92 mM Tris-HCl (pH 7.5), 0.18 M NH₄Cl, 9.2 mM MgCl₂, 0.92 mM dithiothreitol, 0.92 mM GTP, 10 pmol of low-salt-washed ribosomes, oligonucleotide as indicated below and about 1.3 nmol of stripped, unfractionated tRNA loaded with fMet (estimated to contain about 30 pmol fMet-tRNA) in a volume of 13 μ l. After 15 min at 37 °C, 2 pmol of Q β [³H]RNA (○) (specific activity, 1.3×10^5 c.p.m. pmol⁻¹) and 100 pmol of [³²P]pA-U-G (●) (specific activity, 1.9×10^4 c.p.m. pmol⁻¹) were added in 7 μ l and incubation was continued at 37 °C for 10 min. The sample was then analysed essentially as described in Fig. 1. a, No oligonucleotide, plus fMet-tRNA; b, no oligonucleotide, no fMet-tRNA; c, 40 pmol of P-2(a+b), plus fMet-tRNA. Brackets indicate the fractions corresponding to the 70S complex.

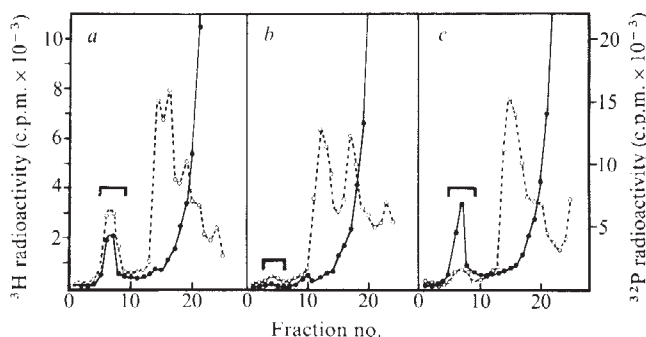


Table 1 Inhibition of binding of 70S ribosomes to Q β RNA by oligonucleotides

Oligonucleotide	Nucleotide sequence (ΔG (kcal mol ⁻¹))*	Amount added (pmol oligonucleotide)	% of Q β [³ H]RNA bound to 70S ribosomes (relative to control†)	% Inhibition
None		none	100	0
P-2a (purified by ribosome binding) ³	A-G-A-G-G-A-G-G-U _{OH} (-16.9)	5	42.6	57.4
P-2a (purified by ribosome binding) ³		10	32.6	67.4
P-2a (purified by ribosome binding) ³		20	12.8	87.2
P-2(a+b) (1:1 mixture)	A-G-A-G-G-A-G-G-U _{OH} (a) (-16.9) G-G-G-A-G-A-A-G-U _{OH} (b) (-10.0)	20	8.8	91.2
P-2(a+b), treated with RNase T ₁ ‡		20	115.0	0
P-4	G-G-A-A-G-G-A-G-C _{OH} (-11.1)	20	43.4	56.6
P-6	G-G-A-G-A-A-G-C _{OH} (-7.9)	20	57.5	42.6
T-14	A-U-U-U-C-U-U-C-U-A-U-G _{OH} (-1.1)	20	105.0	0
T-2	A-U-A-U-U-A-U-U-U-U-A-C-C-G _{OH} (-1.1)	20	90.0	10.0

Low-salt-washed ribosomes (5 pmol) were incubated with oligonucleotides in the conditions described in the legend to Fig. 1 for 15 min at 37 °C, then Q β [³H]RNA (2 pmol, specific activity 6.9 $\times 10^4$ c.p.m. pmol⁻¹ or 3 $\times 10^4$ c.p.m. pmol⁻¹) was added to the mixture and incubation continued for 10 min at 37 °C. The mixture was diluted with 100 μ l of buffer B and analysed by 10–30% sucrose gradient centrifugation as described elsewhere⁵.

* Free energy of optimal interaction with the sequence HOA-U-U-C-C-U-C-C-A-C-U-A-G (5') of the 3' end of 16S rRNA was calculated according to Gralla and Crothers¹⁷, without considering the free energy contribution resulting from the bimolecular nature of the reaction.

† In the control experiment where oligonucleotide was omitted, about 20% of the input Q β [³H]RNA was bound to 70S ribosomes (see Fig. 1, a).

‡ BrCN-activated Sepharose 4B was prepared and RNase T₁ was immobilised essentially as described¹⁵. ³²P-labelled oligonucleotide P-2(a+b) (50 pmol in 5 μ l H₂O; specific activity, 200 c.p.m. pmol⁻¹) was allowed to run into a small column (0.1 ml) of immobilised RNase T₁ and to stand for 15 min at room temperature. The RNA was rinsed out of the column and an aliquot was taken for electrophoretic analysis¹⁶: only A-A-Gp, A-Gp, Gp and Up were detected. Digested RNA equivalent to 20 pmol of P-2(a+b) (as determined by radioactivity) was tested for inhibitory activity as described for the untreated fragments.

than those required with P-2a. Two pyrimidine-rich RNase T₁-resistant oligonucleotides, T-14 and T-22, gave virtually no inhibition at a molar ratio of 10 relative to Q β RNA (Table 1). It is interesting in this connection that AG-rich random copolymers were found to be potent inhibitors of ribosome binding to natural mRNA⁸.

To determine the dependence of pA-U-G binding to ribosomes on fMet-tRNA, a mixture of 5'-³²P-labelled pA-U-G (100 pmol) and Q β [³H]RNA (2 pmol) was incubated with ribosomes (10 pmol) in the presence or absence of fMet-tRNA and 70S complex formation monitored by sucrose gradient centrifugation. As shown in Fig. 2, the binding of Q β RNA as well as of the pA-U-G was strongly dependent on the addition of fMet-tRNA to the incubation mixture: 0.3 pmol of Q β RNA (15% of input) and 0.6 pmol of pA-U-G (0.6% of input) were bound when initiator tRNA was added, and 0.06 pmol were bound in either case when the tRNA was omitted. The binding of pA-U-G was almost proportional to its input level, up to 100 pmol. At 10 pmol, the binding of pA-U-G was 0.08 pmol, about one-quarter that of Q β RNA at an input of 2 pmol. These results may indicate a different rate of complex formation for Q β RNA and pA-U-G; it is less likely that the yield of 70S complex would reflect an equilibrium between free and complexed components in the conditions of our assay, as centrifugation through a sucrose density gradient removes the excess oligonucleotide from the equilibrium. Oligonucleotide P-2(a+b) (a mixture of P-2a and P-2b) inhibited binding of Q β RNA but distinctly stimulated that of pA-U-G (Fig. 2c, Fig. 3) at all levels tested; the values for 40 pmol of P-2(a+b) were 78% inhibition for Q β RNA and 42% stimulation for pA-U-G. The corresponding values for 40 pmol of oligonucleotide P-6 (see Table 1) were 21% and 25%, respectively. The stimulation of binding of pA-U-G by P-2(a+b) may be due to increased availability of ribosomes no longer bound to Q β RNA.

There are great differences in the stabilities calculated for the putative interactions between the 3'-terminal region of 16S

rRNA and the Shine-Dalgarno regions of different mRNAs². It has been proposed that both the Shine-Dalgarno interaction and the interaction between initiator site and fMet-tRNA anticodon have a role in the formation and/or stability of the initiation complex^{2,5} and that one or the other may make the greater contribution, depending on the nature of the mRNA. Several sets of experimental findings bear on this hypothesis. (1) In the case of the Q β coat cistron, where the Shine-Dalgarno interaction is relatively weak ($\Delta G = -7.9$ kcal mol⁻¹), a mutation converting the A-U-G initiator triplet to A-U-A reduces the amount of 70S complex recovered in binding experiments to less than 10% (ref. 5). An A-U-G $\rightarrow$ A-C-G mutation in the cistron for the 0.3 protein of phage T7 (ΔG of Shine-Dalgarno interaction = -10 kcal mol⁻¹) does not diminish the efficiency of ribosome binding (in this case, however, it is not excluded that binding might occur at an A-U-G triplet two codons downstream)⁹. (2) In the presence of fMet-tRNA, *Escherichia coli* ribosomes bind to the coat cistron and to a lesser extent to the replicase cistron, but in its absence only to non-initiator sites which show extensive complementarity to the 3'-terminal region of 16S rRNA ($\Delta G = -11.1$ to -16.9 kcal mol⁻¹)³. Ribosomes bind efficiently, even in the absence of fMet-tRNA, to an RNA fragment from the R17 initiator region of the A cistron, where the Shine-Dalgarno interaction has a ΔG of -15.8 kcal mol⁻¹ (refs 2, 10). (3) The trinucleotide pA-U-G binds to 70S ribosomes, and at least one mRNA, the λ Prm RNA, which carries the initiation triplet at its 5' end and thus lacks the 'Shine-Dalgarno' region, is translated *in vivo*¹¹; however, binding of the trinucleotide pA-U-G seems to be far weaker than binding of Q β RNA as judged by the molar amount of the material retained by ribosomes, and the efficiency of translation of the repressor gene by Prm promoter is an order of magnitude less than that of Pre, the same mRNA preceded by a leader sequence comprising a Shine-Dalgarno region¹². (4) A point mutation diminishing the extent of the Shine-Dalgarno interaction at the initiator region of the 0.3 protein of phage T7 causes a shift of

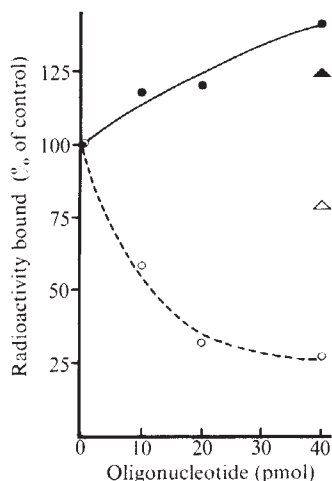


Fig. 3 Effect of oligonucleotide P-2(a+b) and P-6 on the binding of 70S ribosomes to Q β [3 H]RNA and [32 P]pA-U-G. Q β [3 H]RNA and [32 P]pA-U-G were incubated with ribosomes, fMet-tRNA and the amounts of P-2(a+b) or P-6 indicated, and 70S complex formation was determined as described in the legend to Fig. 2. ○, Q β [3 H]RNA and P-2(a+b); ●, [32 P]pA-U-G and P-2(a+b); △, Q β [3 H]RNA and P-6; ▲, [32 P]pA-U-G and P-6.

ribosome binding to an A-U-G located 15 nucleotides downstream, whereas a suppressor mutation, which creates a more stable Shine-Dalgarno interaction at the original site, restores the normal ribosome binding capacity of the mRNA.

If P-2a blocks the formation of Shine-Dalgarno interactions, then ribosomes could only attach to Q β RNA by virtue of the interaction between fMet-tRNA and A-U-G. If we assume that there are no other important interactions between ribosomes and natural mRNA, the rate of formation or stability of complexes between 70S ribosomes and the trinucleotide A-U-G on the one hand and any other mRNA on the other should become similar in the presence of oligonucleotide P-2a. It will be of interest to compare the effect of this oligonucleotide on ribosome binding and protein initiation, both with Q β RNA and with other messengers.

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An intervening sequence of the mouse β -globin major gene shares extensive homology only with β -globin genes

THE mouse β -globin major (β^{maj}) gene is interrupted by two intervening sequences of DNA that divide it into three discontinuous segments¹⁻³. The entire gene, including the coding, intervening and untranslated regions, is transcribed into a colinear 15S mRNA precursor⁴ containing a 5'-cap structure and 3'-poly(A) (refs 5-7). Because mature globin mRNA is significantly smaller (10S) and does not contain these intervening sequences, the 15S precursor must be processed. Such processing presumably accounts at least in part for the reduction in sequence length observed between so-called heterogeneous nuclear RNA (Hn RNA) and cytoplasmic RNA⁸⁻¹⁰. Intervening sequences seem to occur in a variety of genes and organisms^{1,11-18}, but their function and representation in the genome have been unknown.

The availability of cloned β -globin genes^{3,16} permits us to answer certain obvious questions about the structure and occurrence of intervening sequences. Previously, we examined the extent of the homology between cloned β -major and β -minor (β^{min}) genes by means of restriction endonuclease analysis and by visualisation of heteroduplex structures formed between them. Of the 7,000 base pairs compared, shared non-coding sequences comprised only a few hundred base pairs¹⁶. In the longer (646 base pairs) intervening sequence, homology was found only in the regions bordering the structural gene sequences; thus, the central portion of the intervening sequences has diverged. The significance of this conserved homology, perhaps representing retained regulatory signals or sites important for the elimination of intervening sequences, is unknown. Any homology to other portions of the genome has likewise been unknown. Do parts of the intervening sequence constitute a genetic signal retained among unrelated genes throughout the genome, analogous to bacterial insertion

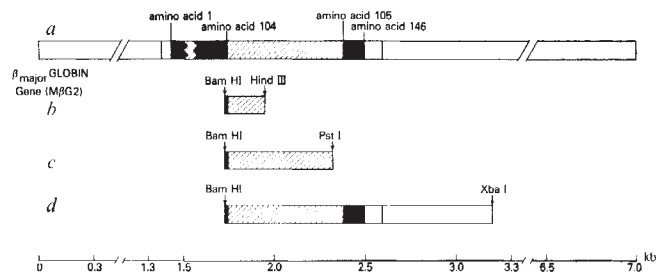


Fig. 1 Derivation of intervening sequence containing probes from β^{maj} (M β G2) DNA. *a*, The M β G2 mouse genomic *Eco*R1 fragment containing the β^{maj} gene, including flanking, transcribed but non-translated, intervening and coding sequences. This fragment was cloned intact into the plasmid PMB9, yielding PMB9- β G2 (ref. 2). *b*, (IVS-A), PMB9- β G2 DNA was digested with *Bam*HI and *Hind*III (New England Biolabs), ligated with similarly digested PBR322 DNA, and used to transform χ 1776 (ref. 2). Clones hybridising the excised 230-base pair fragment were picked, grown up, and the 230-base pair *Bam*-*Hind*III fragment cut out of a 9% polyacrylamide gel, eluted by electroelysis and concentrated on DEAE cellulose. *c*, (IVS-B), PMB9- β G2 DNA was digested with *Bam*HI and *Pst*I, and the appropriate 620-base pair fragment isolated from a 9% gel as above. *d*, (IVS-C), PMB9- β G2 DNA was cleaved with *Bam*HI and *Xba*I and the 1,500-base pair fragment isolated as above except from a 5% gel. □, Genomic flanking sequences; ■, coding sequences; ▨, intervening sequences; ▩, transcribed, untranslated sequences.

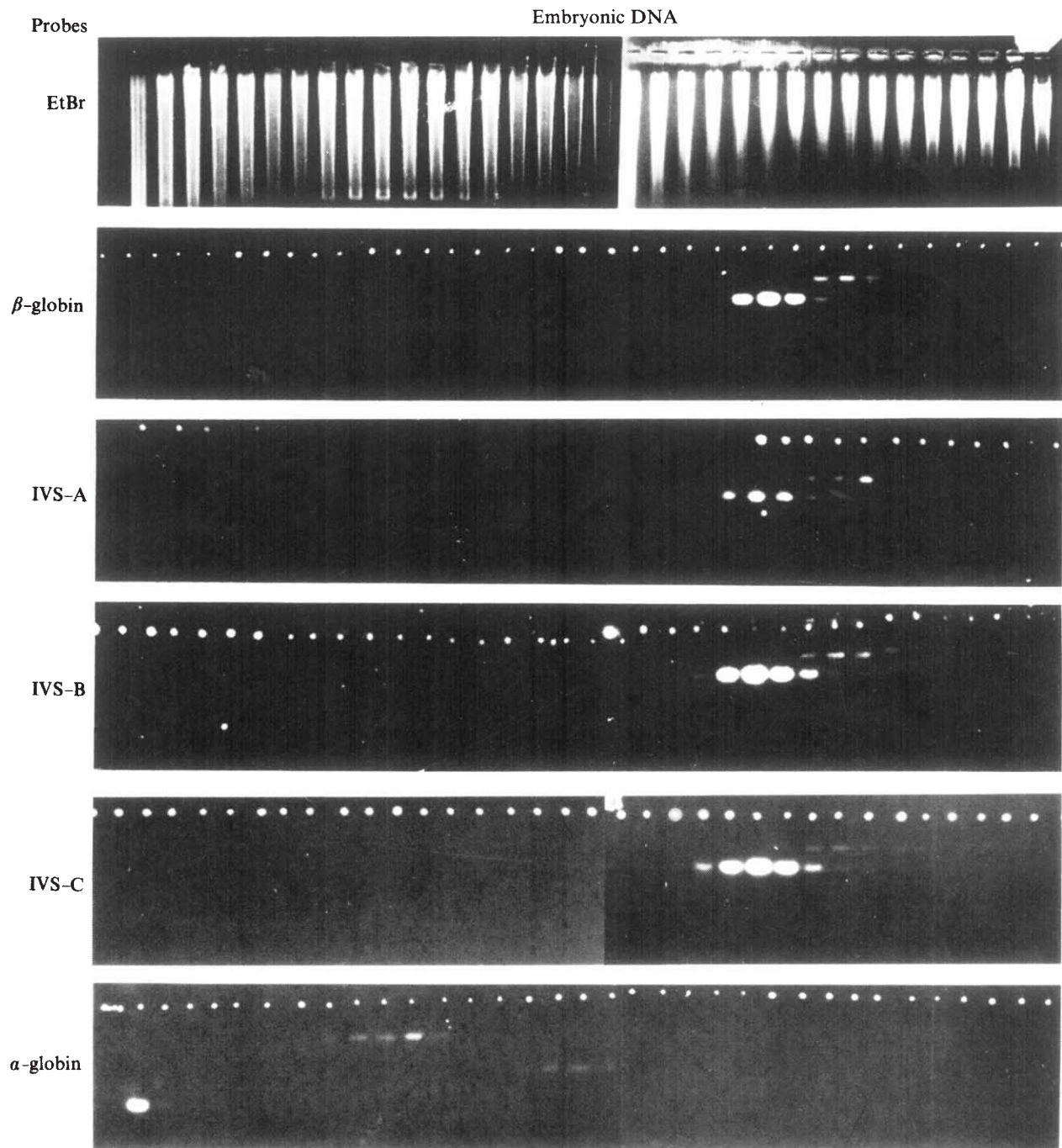


Fig. 2 Hybridisation to genomic DNA of various probes containing intervening sequence from β^{maj} (M β G2). EtBr, an ethidium bromide-stained 1% Agarose gel containing 35 pooled fractions from an RPC-5 chromatograph of *Eco*RI DNA; direction of migration is from top to bottom. The DNA in gels identical to the stained gel shown was transferred to a Millipore nitrocellulose filter¹³ and hybridised to various ^{32}P -labelled probes (see Fig. 1) as follows: ^{32}P -pCR1 $\cdot$ β M9 (β -globin); ^{32}P -IVS-A; ^{32}P -IVS-B; ^{32}P -IVS-C; ^{32}P -pCR1 $\cdot$ α M10 (α -globin). Probes were labelled by nick-translation²² to specific activities of 86 c.p.m., 158, 12.7, 143 and 18 c.p.m. pg^{-1} , respectively.

sequences¹⁹? Are intervening sequences shared among genes expressed coordinately in synthetic pathways, such as those involved in erythropoiesis? Or are intervening sequences completely unique? To answer these questions, we have used an especially powerful form of analysis involving a two-dimensional distribution of *Eco*RI fragments of mouse genomic DNA, RPC-5 chromatography and Agarose gel electrophoresis. The resulting pattern is then transferred to nitrocellulose filters and hybridised *in situ* to ^{32}P -labelled probes²⁰ containing all or part of the β^{maj} intervening sequence. We have examined the

genomic representation of the larger intervening sequence of the mouse β^{maj} gene and report here that it shares extensive homology only with elements of the β^{maj} and β^{min} genes.

Figure 1 shows the original cloned mouse globin gene, β^{maj} (M β G2) (Fig. 1a) and the probes derived from it. The β^{maj} gene is comprised of genomic flanking, coding and intervening sequences, as shown. Preliminary sequencing studies (D.A.K., unpublished) indicate that the 5' terminus of the small intervening sequence begins after the codon for amino acid 30; its precise length has not yet been determined. The first probe, derived

from a sub-clone of M β G2, is a 230-base pair fragment which extends from a *Bam*HI site at amino acid codon 99 to a *Hind*III site near the middle of the intervening sequence; this probe (designated IVS-A) contains the most distal 18 base pairs of the 5' coding sequence and the proximal 220 base pairs of the intervening sequence. The second probe depicted in Fig. 1, a *Bam*HI to *Pst*I fragment of approximately 600 base pairs (designated IVS-B), also contains the distal 18 base pairs of the 5' coding sequence but extends through the intervening sequence to within 75 base pairs of the 3' coding sequence. The third probe, an approximately 1,500-base pair *Bam*HI to *Xba*I fragment (designated IVS-C) contains in addition to the distal 18 base pairs of the 5' coding sequence, the entire intervening sequence, 3' coding sequence, 3' transcribed but untranslated region, and approximately 600 base pairs of genomic flanking sequence.

To identify sequences in the mouse genome homologous to these probes, murine embryonic DNA was purified, fragmented with *Eco*RI and fractionated by RPC-5 column chromatography. Aliquots from the RPC-5 fractions were electrophoresed, blotted on to nitrocellulose paper and then annealed to a ³²P-labelled probe^{16,20}. The results of hybridisations with the three probes are shown in the third (IVS-A), fourth (IVS-B) and fifth (IVS-C) panels of Fig. 2. The identical appearance of these three panels is notable, as is their identity with the second panel (β -globin), where the probe was the ³²P-labelled β -globin structural gene sequence cloned in plasmid pCR1 (ref. 21). The two discrete bands observed with the β -globin structural sequence as probe (and hence, those bands in the same positions in the IVS-A, IVS-B and IVS-C hybridisations) represent the two non-allelic β -globin genes: the upper, less intense band is the 13-kilobase β^{min} (M β G3); the lower, more intense band, the 7-kilobase β^{maj} (M β G2). The last panel (α -globin) demonstrates the band pattern of mouse α -globin sequences; in this experiment, the probe was the ³²P-labelled α -globin structural gene sequence cloned in pCR1 (ref. 21). Note that three α -globin genes are detected and that there is no homology between any of these and the β^{maj} probes (compare IVS-A, B and C to α -globin). The top panel (EtBr) does not depict an autoradiogram but is simply an example of a gel stained with ethidium bromide and photographed before blotting and hybridisation; note the large amounts of DNA of heterogeneous size in each RPC-5 fraction, each lane containing of the order of 10⁴ genomic fragments.

Before drawing conclusions from the above experiments, we should consider the ability of our probes to detect homologous sequences. From a comparison of the partial sequences of M β G2 (β^{maj}) and M β G3 (β^{min}) as well as heteroduplex studies, we are certain of at least 50 base pairs of homology between probes IVS-A or IVS-B and β^{min} (ref. 16; D.A.K., unpublished data); it is evident from Fig. 2 that this degree of hybridisation is detected. We know, however, from unrelated experiments, that IVS-A barely detects an 18-base pair homologous sequence (H.I.M., unpublished data). The minimum size of homologous sequences resolved in these experiments is, therefore, approximately 20–50 base pairs.

We conclude that the long intervening sequence of the mouse β^{maj} gene shares detectable homology in the mouse genome with only the β^{min} gene. We cannot rule out short sequences which might be more widely shared among intervening sequences and serve as recognition sites for processing. It is possible that rather than similarity or identity of primary sequence, there are within the intervening sequences characteristic conformations conferred on the mRNA precursors by secondary and tertiary structures. Alternatively, the sequences surrounding the intervening sequence may provide the specificity required for the elimination reaction. Such a possibility is especially suggested by what is known of yeast tRNA intervening sequences^{12,17}. These differ among tRNA precursors, whereas the conformations of the tRNA molecules themselves are probably quite similar. Further, recent evidence involving a portion of a putative intervening sequence in the chicken ovalbumin gene suggests that it is

present only in the ovalbumin gene¹⁸. Ultimately, a comparison of the structure of all these elements should permit us to define the critical features required for splicing.

Note added in proof: We have recently determined the entire nucleotide sequence of the mouse β -globin major gene (Konkel *et al. Cell*, in the press) and find short closely homologous sequences at the borders of both small and large intervening sequences.

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Errata

In the article 'The tau heavy lepton—a recently discovered elementary particle' by M. L. Perl, *Nature* **275**, 273–278, in line 6 on page 274 for reaction (2) read reaction (3). In line 13 for equation (1) read equation (2).

In the letter 'Viral sequences related to a human skin papillomavirus in genital warts' by G. Orth *et al.*, *Nature* **275**, 334–336, on page 335 right-hand column line 6 should read ... are close to those ...

The title to the letter by Jones, Kindman and Knowles, *Nature* **275**, 564, should read: 'Stereochemistry of phosphoryl group transfer using a chiral [¹⁶O, ¹⁷O, ¹⁸O] phosphate monoester: the stereochemical course of alkaline phosphatase'.

matters arising

Possible Sun-weather correlation

GERETY ET AL.¹ recently repeated part of my work² on the correlation between solar activity and weather for the zones 50°N–60°N and 60°N–70°N. I was not surprised by the fact that the authors have found substantial differences between my results and theirs for the simple reason that the two statistical samples on which the papers were based differ significantly. In fact, in my work I have used only those stations which provided long-term observational data, whereas Gerety *et al.* used stations with observational data even of 1 or 2 yr. Furthermore, in my paper I excluded a few annual values of $R - R_0$ which appeared to differ significantly from the mean annual value. However, despite these remarkable differences between the two statistical samples, Gerety *et al.* have found a general similarity in the variation of the precipitation in the two northern zones. This can be clearly seen in Fig. 1 where the continuous lines represent my calculations and the dashed lines the calculations of Gerety *et al.* One could not expect to obtain a closer correlation from a comparison of two statistical samples so different in the kind and the number of stations used in the cited papers.

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1. Gerety, E. J., Olson, R. H. & Roberts, W. D. *Nature* **272**, 231–232 (1978).
2. Xanthakis, J. *Solar Activity and a Global Survey of Precipitation* (Research Center for Astronomy, Academy of Athens, 1975).

GERETY REPLIES—Although in preliminary analyses stations with only a few years data were included, the study published in *Nature* included no stations with a length of record of <15 yr. To be more specific about the statistical similarity between our separate samples: (1) In the latitude band 60°N to 70°N Xanthakis used 42 stations. Three of these stations had record lengths of less than 15 yr and two were located in another latitude band entirely. Our sample included all of those used by Xanthakis with the exception of the five just discussed and two more for which we could find no data. The total number of stations in our sample was 97. (2) In the latitude band 50°N to 60°N Xanthakis used 71 stations, one of which had a record length of less than 15 yr. Our sample included all but five of the remaining stations. The total number of stations in our sample was 159.

We agree with Xanthakis that there is a “general” similarity between his curves

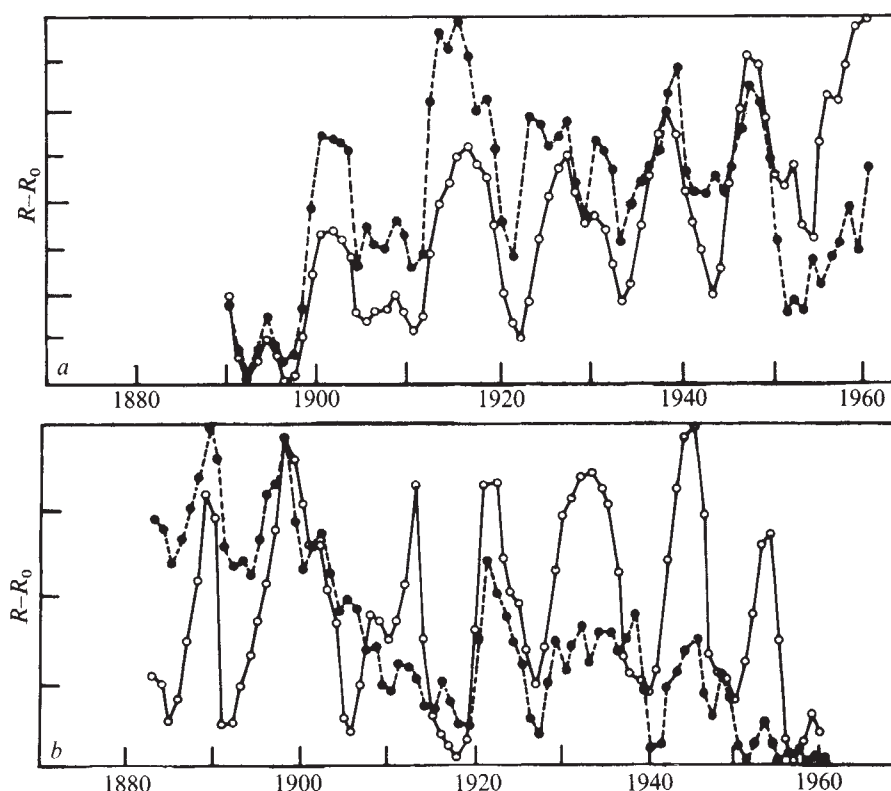


Fig. 1 Comparison of the calculations of Gerety *et al.* (dashed line) and the author (continuous line).

and ours but fail to see any hint of correlation between our curves and solar activity as measured by Xanthakis' index Ia. Finally, if there were in fact a correlation between Ia and precipitation measured in this peculiar manner, it seems to us that it ought not to depend so strongly on one's choice of stations.

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Oxygen in the palaeoaquatic environment

The article by B. and A. Henderson-Sellers has an inappropriate title because it is not concerned with oxygen in the palaeoaquatic environment *per se* but attempts to identify, at the end of the Precambrian, the most likely region on the Earth where diversification of multicellular animals took place, by examining oceanic oxygen levels from the average

position of the density gradient or pycnocline at that time. Thus they show pycnocline depth as a function of latitude, 600 Myr ago, on the basis of a model which depends on several assumptions, the principal being that the palaeocean was density-stratified like the water of a temperate lake during the summer², and from an assessment of the concentration of oxygen above the pycnocline (acting as a barrier to downwards diffusion of oxygen released by photosynthetic organisms) they conclude that multicellular animals originated, and diversified, in latitudinal zones between ~50 and 70° N and S. Their decision to examine the distribution of oceanic oxygen levels 600 Myr ago was evidently influenced by the very late and sudden diversification of life, that “apparently” took place at the end of the Precambrian.

Assuming that 600 Myr ago oxygen was limited to water above the pycnocline, the Henderson-Sellers have suggested only that, at that time, the surface waters of certain latitudinal zones contained a sufficiently high concentration of oxygen to be suitable for the existence of multicellular life. They have not provided evidence showing that such conditions did not exist at the same or different latitudes before 600 Myr ago and, therefore, have

failed to tie the origin and initial diversification of multicellular animals either to the end of the Precambrian, or to particular latitudinal zones. Evolutionary diversification must have been taking place at that time because once initiated, it is continuous. Eucaryotic unicellular organisms dependent on oxygen were living at least 300–400 Myr before the beginning of the Cambrian³, and the fully aerobic environment in which they existed⁴ was equally suitable for multicellular animals. That this was the situation for at least 100 Myr before the end of the Precambrian is shown by the presence of fossils of a variety of soft-bodied metazoans in late-Precambrian rocks in several parts of the world^{5,6}. These late-Precambrian organisms were, however, already complex forms, so that the evolution of metazoans from protists⁴ clearly took place still earlier in the Precambrian, and was not connected in any way with environmental conditions at the end of the Precambrian.

The concept of a sudden diversification of life at the end of the Precambrian derives from the time when it was widely believed (from the lack of fossil evidence) that metazoans evolved only shortly before the beginning of the Cambrian. Thus, Cambrian strata, in contrast to apparently unfossiliferous Precambrian rocks, were known to contain abundant fossils representing the principal animal phyla now inhabiting the world as well as extinct ones. The abundance of fossils at that time was a result of the acquisition of preservable hard parts independently by many groups of organisms. Although the latter was not synchronous, it seemed more nearly so when the Lower Cambrian was believed to represent a period of 30 Myr as against the currently recognised 50 Myr (ref. 7). The complex multicellular organisms that developed hard parts were, however, not living in the surface water of the ocean but on the sea floor, and so they were derived from less complex forms already adapted for a benthic existence. Hence, the sudden diversification of life attributed to the end of the Precambrian or to early Cambrian time refers only to the bottom-dwellers that acquired skeletons, as little is known about the variety of soft-bodied benthic and pelagic animals also living at that time.

Restriction of oxygen, before the beginning of the Cambrian, to surface or near surface water⁸ (above or within the pycnocline) or, in high concentration, to certain latitudinal zones, implies lack of disturbance of the density layers by currents like those of today which move water from one region to another or from one depth to another, and result in a mixing of water and distribution of oxygen to nearly all parts of the ocean. An ocean in which movement of water bodies was too slow to significantly affect the stratification would require a more uniform heat distribution than there is at present; such conditions

could only exist in the absence of continents (differential heating giving rise to complex wind patterns, movement of water bodies, and so on) or even of significant rises on the sea floor, that is, the ocean would have to have been global and of more or less uniform depth⁹. Emergence of continents took place long before the end of the Precambrian^{10,11}, and oceanic conditions would then have been comparable to those existing today. During the Lower Cambrian the world consisted of at least five continents separated by oceans^{12,13}, and in the latter, multicellular animals existed at both high and low latitudes¹². Colonisation of the sea floor and overlying waters took place more than 100 Myr before the beginning of the Cambrian as the oldest known metazoan faunas include both benthic and pelagic forms^{6,15}; some of them lived in shallow water whereas others inhabited relatively deep water⁶. Consequently, as far as the fossil record of Precambrian multicellular animals goes back, oxygen was present in water well below the level of the pycnocline.

If multicellular animals, from the time of their origin until 600 Myr ago, had been restricted to the surface water of certain latitudinal zones because oxygen was not available in sufficient concentration elsewhere in the ocean¹, either near the surface or below the level of the pycnocline, diversification would have been inhibited, as their migration (actively, or passively by currents) into other environments where they could survive and where adaptive evolution could take place would have been impossible.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

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B. AND A. HENDERSON-SELLERS
REPLY—Many of Anderson's points are outside the scope of our paper¹, as the temporal evolution from single- to multicellular life within the evolving ocean-atmosphere system is a wide topic and is discussed in more detail elsewhere². The choice of 600 Myr ago as an example of our model results² was intended to illuminate the discussions of evolutionary processes current in the literature. Many of his points are, of course, still widely contested. However, we attempt to answer the most obvious below. The question of oceanic stratification at our chosen period is still unresolved. Degens and Stoffers³ discuss the anoxic conditions at depth resulting from the chemistries associated with the two layers. Although the permanency of this stratification is in doubt, its certain occurrence lends credence to our model and provides at worst error bars on the timing. Hargraves⁴ postulation of a global sea, although recently criticised⁵, seems to be supported by more recent evidence and discussion^{6,7}.

The existence of a pycnocline, at least for long periods during the ocean's history, implies that the build up of oxygen in the upper layers did occur at times up to and including the diversification and emergence of land animals. Our article was intended to illustrate an additional and important factor in the total description of the complicated process of global evolution. The dynamic feedbacks between life, ocean and atmosphere during the evolution of the Earth will not be simply resolved.

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reviews

Newton's correspondence

I. Bernard Cohen

The Correspondence of Isaac Newton. Vol. VII: 1718–1727. Edited by A. Rupert Hall and Laura Tilling. Pp. 522. (Cambridge University Press: Cambridge and London, 1978.) £25.

WITH this seventh volume, the Royal Society's edition of the *Correspondence* of Isaac Newton, begun under the editorship of H. W. Turnbull (vols 1–3) and continued under J. F. Scott (vol. 4), is now brought to a conclusion. Volume 7 (like vols 5–6) has been edited by A. Rupert Hall and Laura Tilling. The editors observe that very few of the letters included here "may justly be described as scientific". There are exchanges with Varignon and Bernoulli about the calculus, but not its subject matter so much as a rehearsal of the old controversy with Leibniz of a decade earlier over questions of priority in invention. Similarly, an extended set of correspondence with Varignon concerning the *Opticks* deals not with Newton's experiments and theoretical explanations, but rather with the type fount, the details of printing, the illustrations, and so on. There are letters from Pemberton to Newton about the third edition of the *Principia* (1727), which Pemberton was seeing through the press. But Newton's replies are lost and, in any event, most of Pemberton's queries to Newton do not concern fundamentals of mathematics or of physics.

Perhaps the most interesting letters in this volume are those written by or to Pierre Varignon, then a professor of mathematics at the Collège Mazarin and an influential member of the Royal Academy of Sciences in Paris. Varignon was considered by both Leibniz and Johann Bernoulli to be a partisan of the Continental mathematicians, but he "maintained at least a superficial impartiality in the debate", and kept up a friendly contact with both Newton himself and Abraham De Moivre, one of Newton's chief spokesmen. Today's reader may still be astonished by both the heat of the debate and the degree of chicanery and plain dishonesty on the part of Newton, Leibniz, and practically everyone who was in any way associated with the issue. For example, Newton told Bernoulli that he had not "ever taken the trouble of spreading opinions

throughout the world", when he had actually written the draft of the report of the Royal Society's committee to examine the question of the true discoverer or first inventor of the calculus. Nor was that all; on the publication of the committee's documentary report in favour of Newton, the *Commercium Epistolicum*, Newton wrote and published a lengthy (anonymous) summary or 'review' of it in English in the Royal Society's *Philosophical Transactions*. This was translated into Latin and published in a reprint of the *Commercium Epistolicum* with an additional preface (also anonymous) by Newton. As to Bernoulli, he said of a famous letter he had written (also published anonymously), "I am not certain of what kind that letter addressed to Mr Leibniz is of which you speak . . . I do not remember having written to him myself that day, yet I would not deny it altogether . . .".

On a different level altogether is a brief note to Fontenelle, in relation to the presentation to the Royal Academy of Sciences of the second Latin edition of the *Opticks* (1719). Referring to the novelties introduced in this edition, notably the additional Queries, Newton said that "Here I cultivate the experimental philosophy as that which is worthy to be called philosophy" and he added that in his treatise "I consider hypothetical philosophy not as knowledge but by means of queries."

The volume concludes with a discussion of Newton's genealogy and a lengthy appendix, containing additions and corrections to the first six volumes. A considerable part of these addenda deal with Newton's activities as Warden, then Master, of the Mint. It is thus seen that Newton's post "was clearly no sinecure". Not only did he have the difficult job of supervising the construction and operation of County Mints to "receive and recoin the old hammered money", but he took it on himself to attend to the interrogation and prosecution of counterfeiters. These new letters deal with the chronology of Newton's discoveries in the calculus, chemical experiments made by Boyle, and a draft of a proposition for the revised *Principia* (1713) dealing with the resistance experienced by a cylinder that moves through a fluid. In a letter of January 1675, Newton

thanks the Secretary of the Royal Society for the offer of remission of annual dues, explaining that he could no longer afford to remain a Fellow, as "the time draws near that I am to part with my Fellowship, & as my incomes contract." Newton evidently was to resign from his Fellowship at Trinity College in spring 1875, as he could not in conscience go into Orders, as was then required of all Fellows; in April 1675 the Lucasian Professorship—to which he was appointed—was exempted by Royal Patent from the rule about Orders, but for which Newton's professional career would have been severely interrupted.

Scientists and historians and philosophers of science will be grateful to A. Rupert Hall and Laura Tilling on having at last brought to completion the edition of Newton's correspondence, of which the first volume was published in 1959. But any users of this set will regret that the last volume does not contain a comprehensive index to the complete correspondence. Furthermore, the corrections and emendations at the end of vol. 7 apply to all previous volumes, but they neither incorporate the previous list of errata (at the end of vol. 3), nor do they refer the reader to the entries in the earlier list. Thus, every time a letter in vols 1–3 is consulted, the reader must look in two separate lists of errata, which is a great inconvenience. □

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Thin-layer chromatography

Practice of Thin-Layer Chromatography. By J. C. Touchstone and M. F. Dobbins. Pp. 381. (Wiley-Interscience: New York and Chichester, UK, 1978.) \$27.30; £14.50.

PAPER CHROMATOGRAPHY was taken up slowly and with some fear and trepidation by those brave enough to try it at all because neither the paper nor the solvents and reagents were suitable for the technique; and, indeed, it took some time for paper for chromatography to appear on the market. Conversely, thin layer chromatography

took off explosively because, by the time it appeared, most of the general problems of the technique had been solved and it was a relatively simple matter to transfer from paper to thin layers.

If you are about to start chromatography then this book will not describe how to separate the compounds of interest, be they aminoacids, steroids, dyes, and so on. However, if you have some or even a great deal of experience then it is a mine of information covering the whole subject in a most up-to-date manner. Extensive discussions on preparing plates, pre-coated plates, the mobile phase, types of chromatography, documentation, quantification and reproducibility, preparative and other aspects of the techniques are given in a very readable

manner. Many tricks of the trade are described and I found some which I had not picked up in over twenty years of experience. Conversely, I feel that I might add a few to their repertoire. The best way to cut foil-backed layers is with a guillotine and to prevent glass capillaries scratching plates it is best to place 1–2 mm plastic tubing over the end which will be applied to the plate.

This book can be confidently recommended to all who practise thin layer chromatography and it would be a valuable addition to all departmental libraries.

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Animal communication

How Animals Communicate. Edited by T. A. Sebeok. Pp.1128. (Indiana University Press: Bloomington and London, 1978.) £40.

ABOUT ten years ago, T. A. Sebeok edited two books on animal communication, one of which became a standard reference work in the field. The present volume is intended to update and replace both of these earlier ones. Fitting two into one and taking account of progress has led to a massive volume at an astronomical price.

Editing a book to which there are 38 separate contributions must be a daunting task if the contributions are to appear on time and are to be well integrated with each other. Unfortunately, Sebeok has cut corners in these respects, the different chapters varying greatly in style, organisation, degree of detail and length, and many of them (presumably those by the more punctual authors) containing no references after 1974. Strictly imposed word limits tailored to the importance of each author's topic and more rigorous selection of the topics to be covered would have made a shorter, better and cheaper book. This is not to say that the book does not deserve some applause: many of the individual chapters will be, within their limited areas, the definitive reviews and works of reference which the whole book was intended to be.

One reason for its length is that the book is split into three sections, each classifying the field in a different way and thus involving some overlap. The variability of treatment is well illustrated by the first section, which deals with theoretical issues. Here there are three thorough and useful chapters on

evolution (Marler), ontogeny (Burgardt) and action patterns (Barlow). In the other three Lieberman repeats his controversial ideas on the evolution of language, Griffin gives a brief personal perspective and Robertson reviews cellular communication, a topic of little relevance to the book's main theme. The second section deals with communication split up according to the senses and is equally patchy. There are useful conventional reviews of pheromones (Shorey) and visual signals (Hailman), as well as two excellent ones on more specialist but intriguing phenomena: bioluminescence (Lloyd) and electric signalling (Hopkins). Beside these are a more or less irrelevant article on echolocation (Griffin), an out-of-date one on acoustics (Busnel),

reprinted from one of Sebeok's earlier books, and a chapter on tactile communication which deals only with humans (Geldard).

The third section of the book, which accounts for three-quarters of its length, covers communication in selected animal groups in a series of 25 chapters. Some of the groups about which less is known are dealt with almost species by species, yet a more restricted approach has had to be adopted to those which have been more widely studied. Hölldobler shows how well this can be done with his article on social hymenoptera, and the artiodactyls (Walther) and higher primates (Oppenheimer; Gautier and Gautier; Marler and Tenaza) also receive good thorough treatment. The birds lose out a bit with a relatively brief and general article by W. J. Smith. The book ends with an article by Sebeok on the relevance of animal communication to human studies, preceded by one on man-chimpanzee communication by Fouts and Rigby. The editor's view on the rather sterile controversy as to whether the feats of chimpanzees constitute language is neatly expressed by the appearance of the names of these chimps in what would otherwise be an author index.

Overall, this book contains much that is readable and interesting, but it falls short of being an encyclopaedic work of reference because it covers some areas very much more fully than others.

P. J. B. Slater

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Interpreting fossil assemblages

The Ecology of Fossils: An Illustrated Guide. Edited by W. S. McKerrrow. Pp.384. (Duckworth: London; MIT Press: Cambridge, Massachusetts, 1978.) £14; \$22.50.

THIS book is the first attempt to portray fossil communities of marine invertebrates from every geological system since the Precambrian. It contains 125 block-diagrams of fossil assemblages, each picturing animals and/or plants in life-position, in many cases their position when buried as fossils and their appearance in cross-section in the sediment. Each assemblage is described and for each system there is a general introduction. The book owes a great deal to Mrs. E. Winson for her excellent drawings. The fact that different taxa may be impossible to distinguish in these diagrams —

for example, *Orthambonites* and *Pleurothis* in fig.5, or the two species of *Batillaria* in fig.115 — is a useful reminder to the student that fossils cannot usually be identified properly at a glance; but too many of the pictures are given impossibly accurate names.

Although biologists do not agree amongst themselves on how a community can be defined, the authors consider that there is only one way to define fossil communities: a group of animals living in the same habitat. This convenient empirical definition is elaborated in the introduction to the Silurian, and it becomes apparent that they recognise that it is the associations of taxa themselves that define both the habitat and the community living in it: habitat is not intended to be defined by the inorganic facies of the sediment. Nevertheless, many of the communities in this book are tied to particular facies environments — for example, "Upper Devonian Clastic Shelf" or "Muddy Lime Sand" (in the

Jurassic).

The authors recognise that the communities illustrated are a selection from the large number known and that the diagrams emphasise organisms that are common and easily preserved. The implication is still there that the assemblages illustrated are real associations of fossils. This is not always true, and it is noticeable that many of the "communities" are not tied to named stratigraphical units. Thus, fig.85, the Upper Jurassic "Calcarene Community", which from the presence of *Rhaxella* can be presumed to be a Corallian assemblage, is unlike any single example of the 18 detailed benthic associations in the Corallian as described by Fürsich (*Palaeontology*, **20**, 337-385; 1977). Similarly, the *Bostrychoceras* in fig.107 is unknown from the British Maastrichtian chalk "community", unfortunately here called the "Ostrea Lunata Chalk Community" after a species that is actually limited to a very small thickness of the succession that happens to lack many of the other fossils illustrated. These minor mistakes are only part of a general weakness, and perhaps inevitable limitation, of all the reconstructions: they are both unquantitative and subjective. Why include so few ammonites in the Jurassic where they are often abundant, and yet provide an illustration containing seven different species of ammonites in the Late Turoonian Hardground Community (fig.105), which in turn does not include or mention the much more common brachiopods? Why is a *Caninia* not illustrated in fig.50 when it is mentioned in the description of the community? Conversely, *Aviculopecten* is illustrated but we are told nothing of its ecology.

For a book on ecology, the actual amount of ecology given is often very limited, even allowing for the fact that many of the groups of organisms are extinct. For the Devonian, Permian, Triassic and Jurassic there are good attempts at an integrated interpretation of each community, but for many animals in other chapters nothing is given beyond whether it was benthic, planktic or nektonic. Quantitative information is scarce throughout. It is not explained that the linear scales to the diagrams are only to size the organisms, not to indicate the number of specimens that one can expect to find in the rock, nor their original abundance.

Although these weaknesses will be obvious to the professional, the book is likely to be welcomed by students. They will value the novelty of fossils being interpreted in assemblages that they might find for themselves, as opposed to a theoretical discussion of principles or a mere list of facts about

each phylum separately. Although mainly for the British reader, the associations are hardly tied to national frontiers. It would be equally useful elsewhere in northern Europe, and many of the Palaeozoic associations can be found in the eastern part of North America. Moreover, at least an outline is given in many chapters of modifications to be expected in North America and the Tethys. Future editions could be improved by the inclusion of a few famous foreign examples such as the Burgess Shale fauna and a rudist reef association; there could also be more examples of present-day communities, each with a fuller ecological synthesis. Most of all

the bibliography needs to be classified: at present it is no more than a list of the papers referred to. As a result there are three petrological papers by West, but on ecology only one each by Bromley, Fürsich and Kauffman and just two by Seilacher. No reference is made to work by Bulman, Carter, Eager, Hudson, Kirk, Nicol, Oliver, Sohl, Reid or Scott, just to mention some well known names who write in English. The only serious misprint is on p110 where none of the key letters to the diagram has been printed.

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Communication update

On Human Communication: A Review, A Survey and a Criticism. Third edition. By C. Cherry. Pp.374. (MIT Press: Cambridge, Massachusetts, and London, 1978.) £12.25; \$21.85.

THE re-appearance of this book is both timely and welcome. Since the first edition was published the world has seen the emergence and explosive growth of information processing systems of all kinds — not only large-scale electronic computing machines, with associated developments in storage media, data-bases and information retrieval, but many others. Thus we have: satellite and ground-based microwave communication systems that have transformed the world into McLuhan's 'global village', allowing an estimated 0.7×10^9 people to view the late pope's funeral as it took place; new components for the optical transmission and manipulation of information, promising even greater speeds and data densities; private and commercial networks for linking many computers together; large on-line systems for airline reservations and hospital management; and, most recently, developments in component technology that have not only turned computers themselves into mere components, at component prices (so-called microprocessors), but have also allowed the economical production of a host of other 'intelligent' components that have revolutionised systems design in all areas of electronic information handling. Many such systems perform critical functions in society on the basis of information gathered by dialogue with people. By improving and disseminating knowledge of human communication we may hope to avoid such communication disasters as apparently occurred with the signal control system for Britain's M4 motorway in December

1976 when 11 people were killed or injured and the motorway was closed for over 6 hours.

The book is unchanged from the first edition, apart from the addition of one chapter and minor corrections. It is a tribute to the scope and fundamental nature of the book that it survives well this procrustean decision that would have proved fatal to books of lesser stature, but certain sections are consequently rather dated (for example, those touching on machine intelligence and perception, including the machine synthesis and recognition of human speech). Nearly all the shortcomings of the book arise from this source and from failure to update material prefaced by terms such as 'recent' and 'today'.

The book defines communication as an act of sharing, distinguishing sign systems of animals from human language, and symbols from words, noting that language is the basis of rational thought and conduct. It then traces the historical development of our current views on 'information', and goes on to examine and describe language. There is next a review of various methods of analysing speech; but by failing to treat modern developments the author omits mention of Linear Predictive Coding of the speech waveform as well as the Fast Fourier Transform, both of which are highly relevant to computer-based analyses of speech. Then the classical Wiener-Shannon statistical theory of communication is simply explained and the author goes on to show how Bar-Hillel extended notions of information measurement to the semantic domain, based on Carnap's theory of inductive probability. Chapter 7 reviews some of the basic facts and problems of human perception and cognitive activity, ending with the observation that it is the multitude of general concepts and powers of organising them by means of language that form the backbone of human communication, dis-

tinguishing humans from animals.

In the new chapter 8, explaining what is human about human communication, in the light of the recent interesting experiments on communication with apes, there is useful insight (for instance, the idea that classical stimulus-response psychology may be rejected as the sole basis for understanding humans, because such a restriction would rule out human communication and would fail to distinguish one's relationship to a person from one's relationship to a thing). The author also sets down an existentialist view of what it is to be human. However, in excluding computer 'languages' from the domain of true languages—and thus from human communication, preferring to call such constructs coding systems because they require no cognitive effort on the part of the machine — the author touches on an important point about human communication that is not followed up, despite being implied at numerous points throughout the book. The point is that we shall not truly understand human communication until we can understand what kind of cognitive effort is involved; until we can formalise just what changes occur in a receiver's world model, and know how to produce those changes most efficiently, or at least can state what kinds of signal strategies are most likely to be useful. A reference to work by Winograd, Winston, Charniak or Colby would seem relevant here.

The book is somewhat preoccupied with spoken and written symbolic forms of language, at the expense of graphical communication. It is still true that a picture is worth a thousand words, and some forms of dialogue are greatly facilitated by providing for much of the information to be communicated and manipulated in the graphical domain, as instanced by the work of Robert Spence, at Imperial College in London. Indeed, as far as communication between humans and machines is concerned, it is in this area that the greatest progress has been made, perhaps for the same reasons that pictures were used at an early stage in human-human communication. Pictorial representations can have just as rich a complex of syntactic, semantic and pragmatic rules, just as many paralinguistic features of various kinds, as written — if not spoken — forms of what is generally called language; but it seems probable that there is some fundamental difference in the way that picture or spatial reference information is handled by the human.

The general reader will undoubtedly wish to skip the mathematics, mainly in chapter 5, but will find the ground

has been carefully prepared for what follows at each stage of the developing argument. Great care has been taken with the choice of words and the form of expression, and the book is rich with insight and painstakingly collected background material. The historical references, the relevant asides, and the anecdotes are a pleasure and a real aid to understanding for all readers.

Pesticide ecology

Ecology of Pesticides. By A. W. A. Brown. Pp.525. (Wiley: New York and Chichester, UK, 1978.) £17.65.

THE title of this book seems highly relevant to the current interest in 'ecotoxicology'. The author is well-known and respected for his work on resistance of insects to insecticides, a topic that with its strong basis in genetics should lead easily into ecological considerations. In the event, I found the book a sad disappointment.

The first, introductory, chapter gives a brief outline of the main types of pesticide, the economic and medical reasons for their use, their potential hazards to man, and of resistance to insecticides. The bulk of the book, in thirteen chapters, takes insecticides, herbicides and fungicides in turn, and considers their effects on selected components of various habitats. For example, chapter 6 considers insecticides and fish, whereas chapter 8 considers insecticides and birds. This approach makes for a repetitive and superficial account of what could be unifying themes. Inhibition of cholinesterases by organophosphorus insecticides could provide one such theme. Instead, chapter 1 briefly states this as the mode of action, and chapters 6 and 8 give a few field observations on the degree of inhibition that is associated with death. There is little mention of the range of cholinesterases and acetylcholinesterases, and no mention of demyelination. There is no discussion of the possible implications for sublethal effects or of the difficulties in deducing from cholinesterase inhibition whether death is due to insecticide poisoning.

More important, there is a lack of ecology, which should surely mean a consideration of the interactions between pesticides and other factors, and their effects on population size and on community structure. Instead, a large part of these chapters consists of statements of the percentage reduction in population size for species exposed to pesticides in the field, of LD₅₀ and LC₅₀ values, and of residues found in

However, for the serious student of human communication, the book must be regarded as what it is in 1978 — only a beginning. Nevertheless, for pleasure or study, the book will prove a very sound investment.

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tissues. The amount of data is overwhelming, and seems more suitable for reference than for reading. This may reflect the author's prefatory statement that the book metamorphosed from a concise text into a detailed review. The relevance of this material to the understanding of ecological events is difficult to see: when one deliberately kills members of one species changes in other species are bound to occur, irrespective of any direct effects that the pesticide may have on these other species.

At least one ecological principle is invoked, that maximum species diversity gives greatest stability to an ecosystem. This is accepted in chapter 2 without question and without supporting evidence, whereas there are many who would argue the opposite.

Chapters 9 and 10 are devoted to more general themes. The first considers organochlorine insecticides and eggshell thinning. The second considers the movement of insecticide residues, locally and globally. Food chains are considered in some detail, and are shown to concentrate persistent organochlorine insecticides. The account is, however, somewhat partial. Important conflicting evidence is not quoted, and other evidence is not critically appraised. Thus, in one example, concentrations of DDT in predatory fish and birds are given for visceral fat, whereas the concentrations in plankton and small fish are for whole bodies.

There is something of a North American bias in the material considered, with some notable omissions in references to the literature, and readers from other continents may wish that latin names had been given for all species, as well as their common names. These comments aside, the subject index lists both individual pesticides and individual species effectively, and the book could be useful for quick reference to some information on the acute toxicity of pesticides, their persistence and breakdown products.

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CAMAC—system design made easy

Not so many years ago oscilloscopes were being designed and built by research scientists for use in their own experiments. Today such a situation is rare. Oscilloscopes have become standard items that are commercially available but the need for complex electronics to collect data and control experiments has increased. Many research groups are once again facing the problem of designing and building complex data processing systems.

The use of small on-line computers is not a complete solution. A computer is often necessary to handle the volume and rate of data but it must still be connected to the experiment. To make progress in research an experimenter must acquire considerable expertise in computer science and electronics engineering. The problem first became acute in nuclear physics, and a standardised solution was sought. Big science could afford to provide professional support teams to design and build interface electronics and the expertise and experience of these teams have ensured that the solution is tailored to the needs of the research scientist.

The solution is called CAMAC. It has been commercially produced for some years now, it works and is available internationally at a reasonable price. More and more areas of research are turning to computer based systems and the existence of CAMAC ensures that the link between the computer and the experiment can be implemented painlessly.

The following three articles, written by recognised experts in the field, introduce the CAMAC system. They illustrate what CAMAC is, why it is used and how it is used. We hope that this introduction will remove many of the problems often associated with the use of small computers and will ensure that progress in research will be able to take full advantage of progress in electronics.

CAMAC specifications are now available as international and national standards. Further information and details of how to obtain documentation can be obtained from the following:

L. COSTRELL
National Bureau of Standards,
Washington, D.C. 20234

H. MEYER
ESONE/ECA Secretariat
Commission des Communautés Européennes,
CCR-BCM, B-2440 Geel, Belgium

Introduction to the interface standards

Formally, the name 'CAMAC' denotes a set of standards for computer interface equipment. Informally it is applied to 'CAMAC equipment' which conforms to these standards and to 'CAMAC systems' assembled from this equipment. The CAMAC standards were originally developed to meet the needs of large-scale data acquisition systems for nuclear physics research. There have been other large-scale applications in computer control of processes and plant in industry and laboratories.

The following description of CAMAC is concerned mainly with the important but less spectacular field of application to small or medium computer-systems for automated control and data-acquisition in a wide variety of laboratories. On-line systems involve connections between the computer and various control and measuring devices. These may use analogue signals (voltage, frequency, time, pulse-rate, and so on) or digital signals. The connection to the computer must use the particular input-output interface standard adopted by the computer manufacturer. CAMAC offers a powerful and flexible means of matching the differing standards of the computer and the external devices.

The basic principle of CAMAC (Fig. 1) is an assembly of plug-in modular units, one of which is connected to the computer and the others to various external devices and signals. The units are linked by a multi-wire highway (the CAMAC Dataway) which is an integral part of the chassis housing the plug-ins. Each plug-in occupies a multiple of $1/25$ th of the width of the chassis (Fig. 2), and generally has front-panel connections to external devices and signals, and rear connections to the Dataway highway. The Dataway carries signals for digital data and for control of data transfers, and also distributes power. The specification defines the mechanical dimensions of the plug-ins and their interface to the highway in just

sufficient detail to ensure that any plug-in will fit into the chassis and communicate by the highway. It leaves complete freedom to design plug-ins with a wide variety of facilities and external interfaces, and to use appropriate materials, components and styling. A typical highway operation takes $1\ \mu\text{s}$ to address a plug-in module and transfer to or from the computer a data word containing up to 24 bits. This is faster than the sustained data rate of most computers, but slow enough to be technically conservative.

The symmetrical name 'CAMAC' was chosen to symbolise this assembly of plug-ins facing in one direction to the computer and the other to the external devices. The name is now usually assumed to stand for 'computer automated measurement and control', which is a good indication of the main areas of application.

The CAMAC standards were developed by an international group representing users' interests in developing and applying laboratory instrumentation systems. The main standards¹⁻³ and other subsidiary standards were published by the Commission of the European

Communities, and were also published in identical form in the US. They are now being re-issued by the International Electrotechnical Commission and by various national standards organisations.

CAMAC is a standard that encourages manufacturers to produce compatible products which can then be assembled into systems by or for the users. It has led to the commercial production of over 1,000 plug-ins, together with supporting products such as the chassis, by 29 firms in many countries, often with the assistance and involvement of major users.

The main strength of CAMAC lies in the many hundreds of different types of plug-ins that face outwards to the external devices and signals. The functional characteristics and external interfaces of these plug-ins are determined by the users' needs and by market forces, rather than by the CAMAC specification. They range from quite simple units in one module of width through to highly complex units that may occupy two or more modules of width and may include a micro-processor. Some indication of the rich variety of these units can be seen from the following examples:

- General purpose units to accept or deliver digital signals, typically following CAMAC recommended practice for front-panel interfaces based on TTL circuit technology, and so suitable for use with the majority of modern items of equipment.

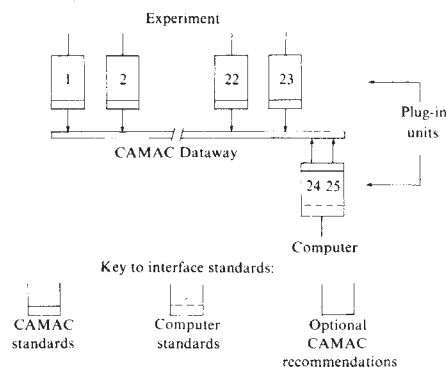
- Units to accept or deliver specialised signals, such as peak-sensing analogue-to-digital converters for the nuclear field.

- Units that are instruments in their own right, such as digital voltmeters in CAMAC units.

- Units that communicate with external instruments, for example through parallel digital interfaces or the General Purpose Instrument Bus.

- Units that are connected by communication links to peripherals such as teletypes or VDUs, or to a host computer.

Fig. 1 Basic small CAMAC system.



Although each of these plug-ins is associated with a particular class of external device or signal it can be used with any computer. The basic costs of the mechanical parts and Dataway interface can be shared by several repeats of the same function within the same plug-in. Thus there are single-width CAMAC plug-ins containing, for example, 12 counters, 16 analogue-to-digital converters, or 8 digital-to-analogue converters.

A wide choice of plug-ins is also available for connecting CAMAC to the computer. Here four different strategies have been adopted, to cover varying requirements for the system configuration: (1) in small stand-alone systems the CAMAC plug-in can include a micro-processor; (2) in small systems with a separate computer, the CAMAC plug-in has an external interface to match the I/O standards of a particular computer (Fig. 1). This arrangement is widely used, and plug-ins are available to match most popular minicomputers; (3) in larger compact systems not exposed to electrical interference the plug-in can be connected to the computer by a CAMAC parallel highway (Fig. 3). This can serve seven CAMAC assemblies, and there are units to couple it to many popular computers; (4) in larger dispersed systems that are exposed to interference the plug-in can be connected to the computer by a CAMAC serial highway (Fig. 3), which can serve 62 CAMAC assemblies.

Although some of these units couple CAMAC to one specific type of computer they can be used with any of the plug-ins that interface to external devices and signals. In typical configurations of CAMAC systems (Figs 1 and 3) all the internal interfaces conform to CAMAC standards, and many of the external interfaces follow CAMAC recommendations.

With falling costs of computers and memory, the computer program specific to a particular application has become a major part of the system cost, especially in 'one off' systems. In any on-line system the software must control input-output through the interface. In the case of CAMAC all I/O operations are controlled by unified commands which specify the plug-in module, a subsection of the plug-in (such as one of the 12 counters, 16 ADCs or 8 DACs

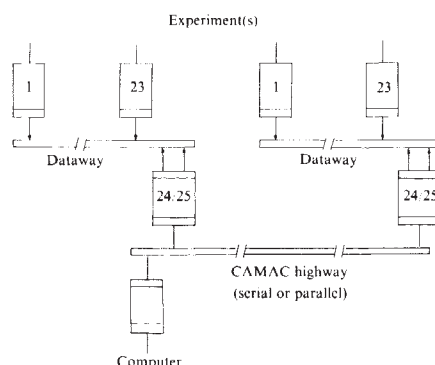


Fig. 3 Larger CAMAC system. See Fig. 1 for key to interface standards.

mentioned above) and the type of operation (such as reading to the computer or writing from the computer). As these commands are uniform for all plug-ins from all manufacturers this helps to simplify and rationalise the software. Several different recommendations for CAMAC I/O software have been published and implemented, to take advantage of the varying needs and skills of the users. First there are recommendations⁴ for macro-instructions for systems programmed in assembly language, an approach often adopted by larger users with appropriate staff. Then there are recommendations for sub-routines⁵, typically to take advantage of the FORTRAN programming experience of scientists. Third, there is an extended form of BASIC⁶ which requires little previous experience of programming or CAMAC. Another derivative of BASIC is well established in the UK for testing CAMAC equipment and for small-scale systems. These four well-documented approaches to CAMAC software provide overlapping coverage of a wide range of situations.

As a multi-national standard that is not tied to any particular firm or type of computer CAMAC has the advantage of a multiplicity of

suppliers, so that the availability of equipment for replacements and extensions is not subject to the commercial policy or survival of any one firm. The wide range of commercially available equipment makes it possible to assemble systems with the minimum of hardware development. At one extreme, the most frequently needed equipment is usually available from several competing suppliers. At the other extreme, equipment to meet some specialised requirement can often be found by searching the published worldwide guide of CAMAC products⁷. Software products are readily available, but generally on an informal or semi-commercial basis from large user organisations.

CAMAC is not a standard that impedes progress—it deliberately allows freedom to exploit new technology such as improved integrated circuits and microprocessors, and to provide the facilities and configurations needed by the user. CAMAC is seen to have considerable advantages in flexibility, versatility, generality and availability when compared with other established methods of interfacing, such as computer manufacturers' interface equipment, specially designed equipment, and the General Purpose Instrument Bus used on many instruments.

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1. ESONE, EUR 4100e (CEC, Luxembourg, 1972).
2. ESONE, EUR 4600e (CEC, Luxembourg, 1972).
3. ESONE, EUR 6100e (CEC, Luxembourg, 1977).
4. ESONE/IML/01 (CEC, Geel, Belgium, 1974).
5. DOE/EV-0016 (Washington DC, 1978).
6. ESONE/RTB/02 (CEC, Geel, Belgium, 1977).
7. European CAMAC Ass. (CEC, Geel, Belgium, 1978).

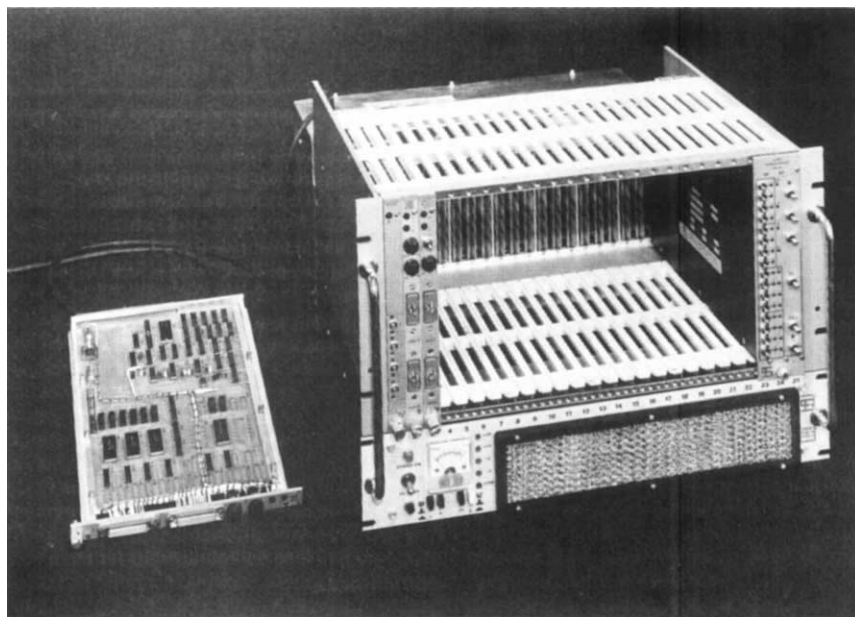
A worked example of system design

Using the CAMAC standards as a basis for laboratory system design, provides practical benefits for the scientist who requires real-time computing power in his research. This can be illustrated by working through the design and implementation of a specific example.

The particular requirement concerned a data acquisition system for a gamma camera, for a large hospital in Belgium. The primary function of the system was to interface to the camera which produces two analogue pulses that have to be amplitude analysed, and the resulting data transferred to a host processor 150 m away. The processor's task was to generate full colour graphic displays of the collected data, and the design of the acquisition system permitted dynamic studies of the camera output to be made with far better time resolution than previously possible. The overall system now gives the doctors not only an improved diagnostic capability on existing camera measurements, but also provides new diagnostic performance on dynamic studies, at the same time generating very significant savings in operating costs over the present photographic methods of recording camera data.

The acquisition system plays only a small, albeit crucial, role in the overall system. It must therefore not only meet the specific

Fig. 2 CAMAC chassis and plug-in units.



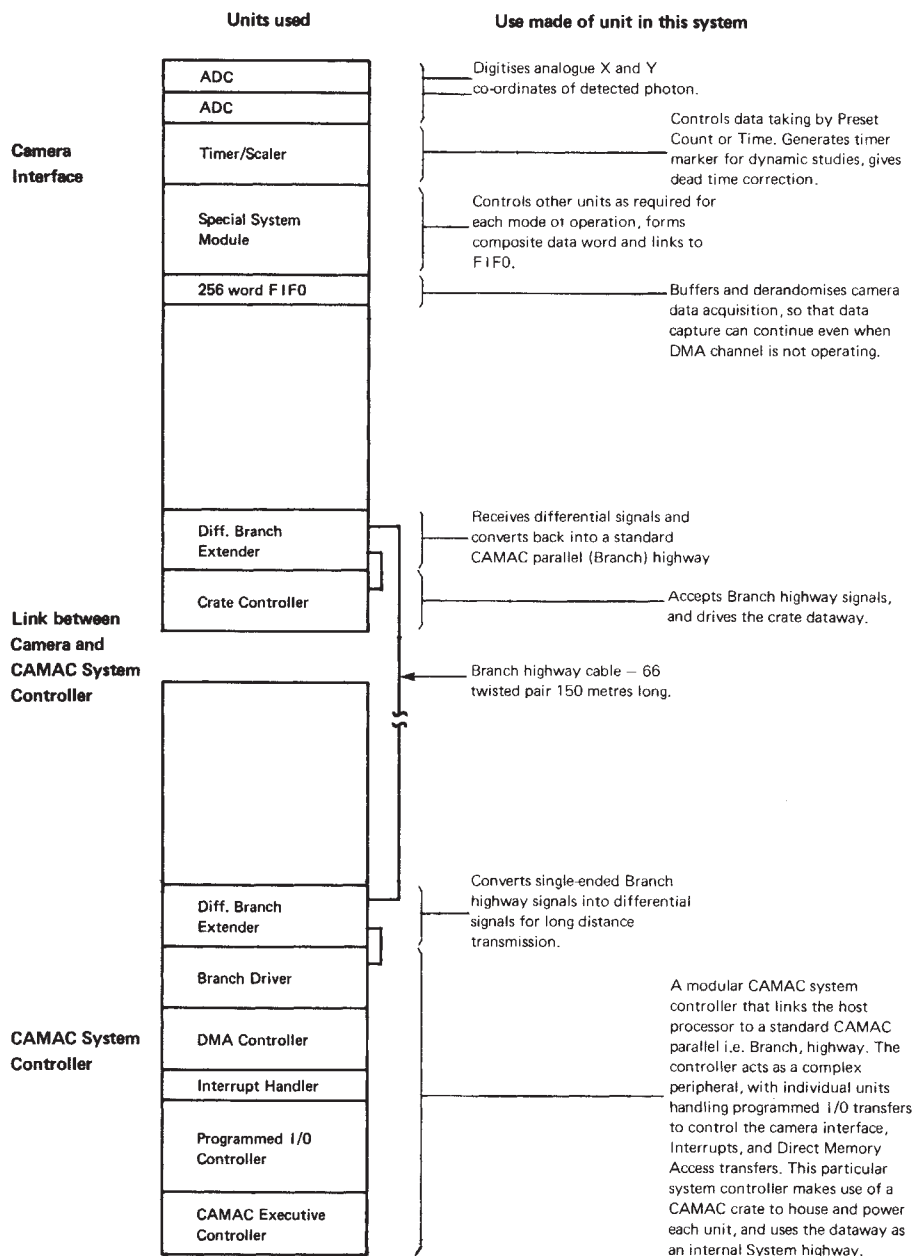


Fig. 1 Block diagram of gamma camera data acquisition system.

performance objectives, but must also be easy to use, maintain and develop as new requirements emerge, whilst minimising costs and implementation time scales at all times.

The starting point in the design of a system to meet this requirement was to base it on CAMAC equipment. In this specific case the decision proved to be readily justifiable, as the system was implemented almost entirely from standard CAMAC products. This was not possible with any other interface scheme. One other approach often considered is the design and construction of a special purpose system. Such an approach raises many fundamental questions of system structure, such as the size of the basic module, the power supply requirements, the way in which each unit relates to the others and so on, none of which have direct relevance to implementing the actual system, but all of which can have a vital impact on the ease with which the system is commissioned, used and maintained, and its flexibility in

coping with new requirements. CAMAC provides a rigorously defined system framework within which the designer is free to concentrate on solving the specific problems posed by the requirements, whilst at the same time building in flexibility into the basic design process. The special design approach was in fact ruled out by the hospital, as they neither had, nor wanted, the level of electronic expertise required to implement a custom-built system. The block diagram of the system is shown in Fig. 1, and divides naturally into three parts; the camera interface, the computer interface, and the link between the two. The requirements for connecting to the camera were very particular to both the specific model of camera used, and the types of measurements required from the system. However, most of this unique requirement was met by a combination of currently available CAMAC units. The special unit acts mainly as a mechanical interface for the cabling between the other units. It contains

only about 20 integrated circuits that control the interconnections in the manner required to achieve the overall interface function. Note that units made by four different companies are used. The system designer is not restricted by the competence of any one supplier. Indeed it is not unusual to find one CAMAC supplier with special competence in analogue unit design, whilst another specialises in the rather different, but equally complex discipline of computer interfaces to CAMAC. Total control of the camera interface is through the CAMAC Dataway, and so it can in principle be controlled by any CAMAC Dataway controller, from a microprocessor resident in the same assembly, to any of the system configurations the CAMAC standards have defined.

The computer interface requirements for this system were more complex than for many laboratory systems. In addition to the normal programmed I/O to control the data acquisition, data transfers have to be at high speed, directly into the host computer's store, and in either List mode (one word at a time transferred into successive memory locations) or in Spectrum mode (the data word is used to address a memory location, whose contents are automatically incremented). Even so the system could be constructed entirely from available CAMAC units, and needed no special design effort. The benefit can be more readily appreciated by the realisation that the unit design effort embodied in just this part of the system represents several man-years of work. Finally the standard CAMAC parallel highway, extended by readily available extender units, was used to provide the required highspeed link over the 150 m between the computer and the camera. For the majority of the units selected in the whole design, the system designer had a choice of several different suppliers. For example, the Crate Controller could have been obtained from at least 10 other companies. Neither would the system design have been significantly affected had the host processor been one of a very substantial range of other types, as most of the system is independent of the computer type, and suitable computer interfaces to the CAMAC parallel highway are available for many different models of computer. That it was possible to meet this somewhat unusual system requirement using available CAMAC units, is a good indication of the scope and range of CAMAC products that are now available to the system designer.

The 'acid test' of any system structure is the ease, or otherwise, with which individual elements designed to be compatible with the system can in practice be combined into a working system. Experience over the past eight years has shown the CAMAC standards to be soundly based, with the integration of individual units into a working system remarkably free of those 'unexpected' interactions that can often affect less completely specified structures. In the case of the camera application this was demonstrated by the ease with which the various manufacturers' units were incorporated. System commissioning was also greatly assisted by the availability of system test and diagnostic equipment, and particularly by the availability of system test software for most of the individual units. The only special test software required was a set of short programs to establish that the system performed the overall functions correctly. All of the diagnostic and test software is written in a high-level BASIC-like language, enabling the user to take advantage of these packages for system maintenance.

The lack of these support facilities can extend commissioning and fault-finding times very significantly.

As a direct consequence of employing commercially available products, the documentation on the individual units is usually to a high standard. The system designer is left with the relatively straightforward task of describing the interaction between these items, and the overall system functions. Good documentation, however, is essential if a system is to remain serviceable in the medium term. It is not uncommon to find equipment that has had to be scrapped once the originator was no longer available to maintain it, because the documentation was never produced. Of course, problems in system design do not end with the commissioning of the system, even assuming that it does fully meet the user's requirements. The system has to be kept in service, and so ease of maintenance can be an important system design consideration. Certainly this was true of the camera system, and the CAMAC approach addresses this problem in several ways. For example, the structure is modular along functional lines. This simplifies the isolation of a fault to a particular unit, and allows first-line maintenance by unit replacement. The system-test software and hardware mentioned above also simplify this process. Faulty units can usually be returned to the supplier for rectification, thus taking advantage of their sophisticated automatic test equipment. As CAMAC equipment is often sold as individual products to system builders, items are often available either 'off the shelf' or on short delivery, and this can act as a valuable back-up to the normal system spares.

Initial system design often makes little or no allowance for the further development of the system. With the CAMAC approach the flexibility to cope with the needs for expansion and up-grading is built into the system structure. Since the initial camera system was commissioned, several additional requirements have been considered, that illustrate this point. The first is to connect the system to three other cameras over the next year or so. As the computer interface will support up to 49 CAMAC chassis, there is in practical terms no physical limit to the number of cameras that could be connected. The second is possibly more interesting, in that it demonstrates how the CAMAC standards have managed to evolve and anticipate new system requirements. As more cameras are added, the host processor will not be able to cope with the instantaneous data rates being generated, and the intention is to add local processing to each camera interface to preprocess the data. The most recent CAMAC standard covers exactly this requirement, and microcomputers are already available that will go into the camera interface assembly and provide the required function.

One final benefit that came from the use of CAMAC in this system was the genuine assistance the hospital staff were able to get from the CAMAC user community in Belgium, in terms of providing basic training and familiarity with CAMAC.

I thank Professor C. Beckers and M. Steels of the Department of Nuclear Medicine, of the Hospital UCL-St Luc, for presenting this interesting problem for us to solve and the directors of GEC-Elliott Process Automation Limited for permission to publish this article.

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CAMAC applications in biological systems

The application of CAMAC to biology has been given an impetus in recent years by the increasing use of large scale experimental facilities producing neutron or photon probes. Data rates may be high and to make the best use of these facilities it becomes of paramount importance that experiments should be highly automated.

From the point of view of such laboratories CAMAC provides a very good solution to several requirements. The systems must be flexible as they may be required to adjust to new experiments every few days so that the hardware must clearly be modular. It must also be largely available commercially because, though there will always be special units that must be designed 'in-house', the total quantity of units required will usually exceed the local design and production capacity of the laboratory. Maintenance problems are simplified as is software implementation as the use of a standard forms a well understood bridge between the programmer or maintenance engineer and the application.

These are general points that apply to systems outside biology as well as within it. Furthermore, though they may seem to be less pressing, these considerations arise also for the individual experimenter implementing a system at his home laboratory.

Several laboratories are using CAMAC to support experiments in biology. At the high flux neutron reactor of the Institut Laue Langevin (ILL) at Grenoble CAMAC output registers are in use for positioning stepper and servo motors on diffractometers and monochromators. Small Angle Scattering (SAS) cameras at ILL use incrementing CAMAC memories to interface 2-D detectors with up to 128 × 128 cells.

The European Molecular Biology Laboratory (EMBL) is standardising the use of CAMAC and it is in use at the main centre at Heidelberg and their outstations at the electron synchrotron DESY at Hamburg and the ILL. Among its present and planned uses are for experiments on fly flight dynamics, EXAFS (extended X-ray absorption fine structure) experiments, centrifuges, stepper motors and polar coordinate X-ray detectors. Three film scanners are interfaced by CAMAC to two Nord-10 computers and a PDP-11/45 and two film writers to Nord-10s. At the DESY outstation the CAMAC serial highway is used to connect two experimental areas separated by some 300 m.

On the electron synchrotron NINA (now closed) at Daresbury, monochromators were also positioned by CAMAC stepper motor drivers and monitored by shaft encoders. Incremental encoders were interfaced by CAMAC up-down binary counters and absolute encoders by input registers. CAMAC 24-bit pre-set scalars were used to record data from ion chambers on EXAFS experiments. An electron storage ring, known as the SRS, is being constructed at Daresbury as a dedicated source of synchrotron radiation, and it is due to become operational in Spring 1980. The data acquisition system will use CAMAC (as does the control system). Among the instruments planned for the SRS are multiple solid-state

detectors. They are being made for energy-dispersive SAS experiments and high rates (100K counts s⁻¹) are desired from each detector. It is intended that incrementing CAMAC memory be used for the data accumulation.

The MRC (Medical Research Council) at Cambridge is developing an experiment using CAMAC to study the dynamic response of muscle to a stimulus. The magnitude of intensity peaks on a linear position sensitive detector will be studied over the 200 ms or so duration of a twitch with the aid of 100 buffered scalars. The scalar buffer registers will be read out every millisecond into a 32K word CAMAC memory while counting proceeds. To achieve the required high rate of transfers on the CAMAC dataway a simple auxiliary crate controller will be used to transfer data from the scalars to the memory.

We hope that these few examples have shown that the same units, stepper motor drivers, memories and scalars, for instance, are relevant to several different experiments. Note too that in CAMAC these units are computer independent it is apparent that an experimenter wishing to set up a new instrument or experiment will find that most if not all of the interfacing problems are already solved. As more systems are implemented in CAMAC the number of shareable and transportable solutions to problems in experimental biology will continue to grow. Daresbury intends to encourage this by loaning CAMAC systems to prospective users of the SRS. They may then develop instruments and experiments in advance of bringing them to the SRS and establish systems at their home laboratories which are compatible with those at the SRS.

To complement the ease with which CAMAC systems may be configured it is highly desirable to provide software that may be as easily written. CAMAC assists here too as the use of standard hardware promotes standard hardware-handling facilities in the software. Several different approaches to this have been adopted. At Daresbury a version of BASIC has been developed with extensions to drive CAMAC efficiently. This will make it possible for the biologist himself to write the programs to control his experiment. The extended BASIC compiler, called CATY, is also in use at EMBL, DESY enabling the two laboratories to share programs. This has happened in the case of EXAFS experiments even though different computers are in use.

As CAMAC finds application in the major facilities, techniques and equipment will evolve that match the many new applications in the area of biological science. This will lead to the availability of equipment that will be relevant also to the small biology laboratory and the scientist will be able to take advantage of the application of computing and modular electronics. This should enable scientists to use data acquisition and experiment control systems employing mini and micro computers in the study of biology without having to devote a significant proportion of their time to problems of electronics.

We thank Drs P. N. Clout of EMBL, R. Ghosh of ILL and A. R. Faruqi of the MRC, Cambridge for providing information used here.

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announcements

AWARDS

The Columbia University's 1978 Louisa Gross Horwitz Prize has been awarded to **David H. Hubel** and **Torsten N. Wiesel** (Harvard University), and **Vernon B. Mountcastle** (The Johns Hopkins University), who will share the award (\$25,000) equally for their contributions to our understanding of the brain.

The Institute of Food Science and Technology has awarded the Mounfield Prizes to **S. R. Tomlinson** (National College of Food Technology) and **Dut Fan Chung** (Polytechnic of the South Bank).

A limited number of Research Fund grants, each of £200, will be awarded by the Chemical Society for the year 1979. Application forms, together with the regulations governing the Fund, may be obtained from Dr Malcolm D. Robinson, The Chemical Society, Burlington House, London W1, UK. The closing date for applications is 1 November 1978.

The Fifth Marconi International Fellowship Award will be given to a person who has made outstanding advances in satellite and space technologies relevant to improving world communications, according to Walter Orr Roberts, Fellowship Secretary. Nominations should be submitted before 15 November, 1978, to the Marconi International Fellowship, Aspen Institute, 1919 14th Street, No. 811, Boulder, Colorado 80302.

MEETINGS

4 December, **Prospects for Power from Ocean Waves**, Edinburgh (Executive Secretary, The Royal Society of Edinburgh, 22 & 24 George Street, Edinburgh, UK).

5 December, **Vibrational Spectroscopy in Inorganic Chemistry**, London (Stanley Langer, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

6-7 December, **The Evolution of Adaptation by Natural Selection**, London (Executive Secretary, The Royal Society, 6 Carlton Terrace, London SW1, UK).

9 December, **Tay Estuary Symposium**, Dundee (Dr J. McManus, Department of Geology, The University, Dundee, UK).

12-13 December, **The Middle Atmosphere as observed from Balloons, Rockets and Satellites**, London (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1, UK).

9-11 April 1978, **Metal Ion Transport and Storage**, Sheffield (Professor P. M. Harrison, Department of Biochemistry, University of Sheffield, Sheffield, Yorks, UK).

17-21 April, **Special FEBS Meeting on Enzymes**, Dubrovnik (Secretariat: Laboratory of Biochemistry, Technological Faculty, Pierottijeva, 6/VI, YU-41000 Zagreb, Yugoslavia).

20-21 April, **Recent Advances in the Study of British Flora**, Manchester (Miss L. Shore, Department of Botany, The University, Manchester, UK).

28 April-2 May, **81st Meeting of the American Ceramic Society**, Cincinnati (R. L. Berger, 3211 Civil Engineering Building, University of Illinois, Urbana, Illinois 61801).

7-9 May, **The Role of Peptides in Neuronal Function**, Bethesda (Dr Barker, Building 36, Room 2C02, NIH, 9000 Rockville Pike, Bethesda, Maryland 20014).

20-21 June, **New Horizons in Industrial Microbiology**, London (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1, UK).

27-31 August, **The Role of Water in Urban Ecology**, Amsterdam (K. C. Plaxton, PO Box 330, Amsterdam, The Netherlands).

2-7 September, **7th International Meeting of the International Society for Neurochemistry**, Jerusalem (Marco Trabucchi, Ente Universitario Lombardia Orientale, Istituto di Farmacologia e Terapia, Via Valsabbina, 19, 25100 Brescia, Italy).

3-5 September, **Erosion by Liquid and Solid Impact**, Cambridge (5th International Conference on Erosion by Liquid and Solid Impact, Cavendish Laboratory, Madingley Road, Cambridge, UK).

3-6 September, **EMAG 79**, Sussex (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3-7 September, **Insect Neurobiology and Pesticide Action**, York (The Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

5-9 September, **15th International Congress of Therapeutics**, Brussels (Secretariat of the Congress, Department of Cardiology, Hôpital Universitaire Saint-Pierre, rue Haute 322, 1000 Brussels, Belgium).

10-13 September, **1st International Symposium on Neuroactive drugs in Endocrinology**, Milan (Eugenio E. Müller, Department of Pharmacology, University of Milan, 32 Via Vanvitelli, 20129 Milan, Italy).

12-13 September, **How will tomorrow's microprocessor based process instrumentation communicate?** London (Miss Audrey Mills, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

16-21 September, **4th Conference on Photographic Gelatin**, Oxford (H. A. Adam, Processing Technology Laboratory, Kodak Ltd, Headstone Drive, Harrow, Middlesex, UK).

17-20 September, **2nd International Reindeer/Caribou Symposium**, Roros (E. Reimers, Institute of Zoophysiology, University of Oslo, Box 1051 Blindern, Oslo 3, Norway).

17-21 September, **7th International Congress of Pathology**, Valencia (Professor A. Llombart-Bosch, Department of Pathology, Medical School, Valencia Apartado 2045, Spain).

19-21 September, **4th National Quantum Electronics Conference**, Edinburgh (Dr B. S. Wherrett, Physics Department, Heriot-Watt University, Edinburgh, UK).

25-27 September, **Organisation of Macromolecules in the Condensed Phase**, Cambridge (Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK).

The previous list of meetings was in *Nature* of 19 October, page 679.

Reports & Publications

UK & Ireland—March

University of Bristol: Department of Agriculture and Horticulture. Long Ashton Research Station, The National Fruit and Cider Institute—Report 1977. Pp.xvi+243. (Long Ashton, Bristol: Long Ashton Research Station, University of Bristol, 1978.) £2.50. [1910]

University College London. Calendar, 1978-1979. Pp.238. (London: University College London, 1978.) [1910]

Social Science Research Council. Research Supported by the Social Science Research Council 1978. Pp.vi+440. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1978.) [1010]

The Nuffield Foundation. Report for the year 1977. Pp.113. (London: The Nuffield Foundation, Nuffield Lodge, Regent's Park, NW1, 1978.) [1010]

Open Earth, No. 1, October 1978. Edited by Dr. Peter J. Smith. Published quarterly. Pp.1-52. (Hanslope, Milton Keynes: *Open Earth*, 32 St. James Close, 1978. Subscription rates available from above address.) [1010]

Liquid Fuel from Coal. (A National Coal Board Report prepared by Planning, Assessment and Development Branch of the Coal Research Establishment, with Operational Research Executive.) Pp.70. (London: National Coal Board, Hobart House, Grosvenor Place, SW1, 1978.) £7.50. [1010]

Employment and Training in the Motor Vehicle Industry. By Peter Brayshaw and Jane Cook. (EITB Reference Paper.) Pp.116. (Watford: Engineering Industry Training Board, 54 Clarendon Road, 1978.) [1010]

Tropical Products Institute Seminar on Post-Harvest Grain Losses. Edited by Mrs. S. P. D. Stokes. (Tropical Stores Products Information, No. 36.) Pp.xvii+72. (Slough: Tropical Stores Products Centre, Tropical Products Institute, London Road, 1978.) £1.60. [1610]

British Standards Institution. Annual Report, 1977 to 1978. Pp.16. (London: British Standards Institution, 2 Park Street, W1, 1978.) [1610]

Food, Health and Farming: Reports of Panels on the Implications for UK Agriculture. Edited by C. J. Robbins. (CAS Paper No. 7.) Pp.119. (Reading: Centre for Agricultural Strategy, University of Reading, 2 Earley Gate, 1978.) [1810]

Scottish Marine Biological Association. Annual Report for the year ending 31st March 1978. Pp.89. (Oban, Argyll: Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, 1978.) £1. [1810]

University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1977-1978. Pp.89-111. (Oxford: The University, 1978.) 20p. [1810]

British Nutrition Foundation. Annual Report, 1977-1978. Pp.24. (London: British Nutrition Foundation, 15 Belgrave Square, SW1, 1978.) [1910]

Other countries—March

Proceedings of the Nutrition Society of Australia. Vol. 3: Third Annual Conference, Adelaide, South Australia, August, 1978. Pp.111. (Blacktown, N.S.W.: Secretary, Nutrition Society of Australia, CSIRO, Division of Animal Production, PO Box 239, 1978.) A\$5. [510]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2046: Calibration of a Mathematical Model of the Antelope Valley Ground-Water Basin, California. By Timothy J. Durbin. Pp.v+51+13 plates. (Washington, DC: US Government Printing Office, 1978.) [510]

Académie Royale de Belgique. Mémoires de la Classe des Sciences. Collection in-8—2^e Série. T. XLIII, Fasc. 1: Nouvelles Recherches sur les Manifestations Vibratoires des Interactions entre Molécules ou Groupes d'Atomes dans les Solides. Par M. J. Pottier. Pp.74. T. XLIII, Fasc. 2: Contribution à l'Étude du Transport Électrique dans les Alliages de Métaux de Transition et de Métaux Nobles à Forte Teneur. Par Michel Brauwiers. Pp.112. (Bruxelles: Académie Royale de Belgique, 1978.) [910]

Bulletin of the American Museum of Natural History. Vol. 161. Article 1: Type Specimens of Birds in the American Museum of Natural History. Part 2. By James V. Greenway, Jr. Pp.1-306. (New York: American Museum of Natural History, 1978.) \$20.45. [910]

Fisheries and Environment Canada: Fisheries and Marine Service. Technical Report No. 785: Physical Characteristics of Atlantic Salmon Spawning Gravel in some New Brunswick Streams. By R. H. Peterson. Pp.iv+28. (St. Andrews, N.B.: Biological Station, 1978.) [1210]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 16079: Ecotoxicology of Chlorpyrifos. By W. K. Marshall and J. R. Roberts. Pp.314. (Ottawa: National Research Council Canada, 1978.) \$2. [1210]

Geological Survey of Canada. Bulletin 266: Carboniferous Biostratigraphy and Correlation, Northwestern British Columbia and Southeastern District of Mackenzie. By E. W. Bamber and B. L. Mamet. Pp.65 (6 plates). Bulletin 267: Contributions to Canadian Paleontology (five papers). By B. S. Norford, et al. Pp.75 (14 plates). \$5.10. Bulletin 276: Stratigraphy and Correlation of Lower Paleozoic Formations, Subsurface of Cornwallis, Devon, Somerset, and Russell Islands, Canadian Arctic Archipelago. By Ulrich Mayr. Pp.55. \$6. Bulletin 302: Devonian Stratigraphy, West-Central Ellesmere Island, Arctic Archipelago. By H. P. Trettin. Pp.119. \$6. (Ottawa: Geological Survey of Canada, 1978.) [1210]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15391: Effects of Arsenic in the Canadian Environment. Pp.349. (Ottawa: National Research Council Canada, 1978.) \$2. [1310]

New Zealand: Department of Health. Environmental Radioactivity Annual Report, 1977. Pp.12. (Christchurch: National Radiation Laboratory, PO Box 25-099, 1978.) [1310]

Studia Forestalia Suecica, Nr. 145: Ultrastructural Studies of Resistance Mechanisms in *Pinus sylvestris* L. Against *Melampsora pinitorqua* (Braun) Rost. (Pine Twisting Rust). By Alena Jonsson, Monica Holmwall and Björn Wallen. Pp.28. (Uppsala, Sweden: The Swedish University of Agricultural Sciences, College of Forestry, 1978.) [1710]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Mechanical Engineering. Report on Research 1975-77. Pp.80. (Melbourne: CSIRO, 1978.) [1710]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2047: Geology and Ground Water in Door County, Wisconsin, with Emphasis on Contamination Potential in the Silurian Dolomite. By M. G. Sherrill. Pp.v+38+5 plates. Professional Paper 950: "Nature to be Commanded...": Earth-Science Maps Applied to Land and Water Management. By G. D. Robinson and Andrew M. Spieker. Pp.95. (Washington, DC: US Government Printing Office, 1978.) [1710]

United States Department of the Interior: Geological Survey. Bulletin 1443: Feasibility and Cost of Using a Computer to Prepare Landslide Susceptibility Maps of the San Francisco Bay Region, California. By Evelyn B. Newman, Arthur R. Paradis and Earl E. Brabb. Pp.iii+27+1 plate. Professional Paper 813-M: Summary Appraisals of the Nation's Ground-Water Resources—Hawaii Region. By K. J. Takasaki. Pp.iv+29. (Washington, DC: US Government Printing Office, 1978.) [1810]

Principles and Methods for Evaluating the Toxicity of Chemicals. Part 1. (Environmental Health Criteria, 6). Pp.272. Published under the joint sponsorship of the United Nations Environment Programme and the World Health Organization. (Geneva: WHO; London: HMSO, 1978.) Sw.fr.28; \$15.40. [1910]

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